Potential harmful cyanobacteria in drinking water reservoirs of Ho Chi Minh City, Vietnam - toxicity and molecular phylogeny.

Christensen, Sara; Daugbjerg, Niels; Moestrup, Øjvind; Annadotter, Helene; Cronberg, Gertrud

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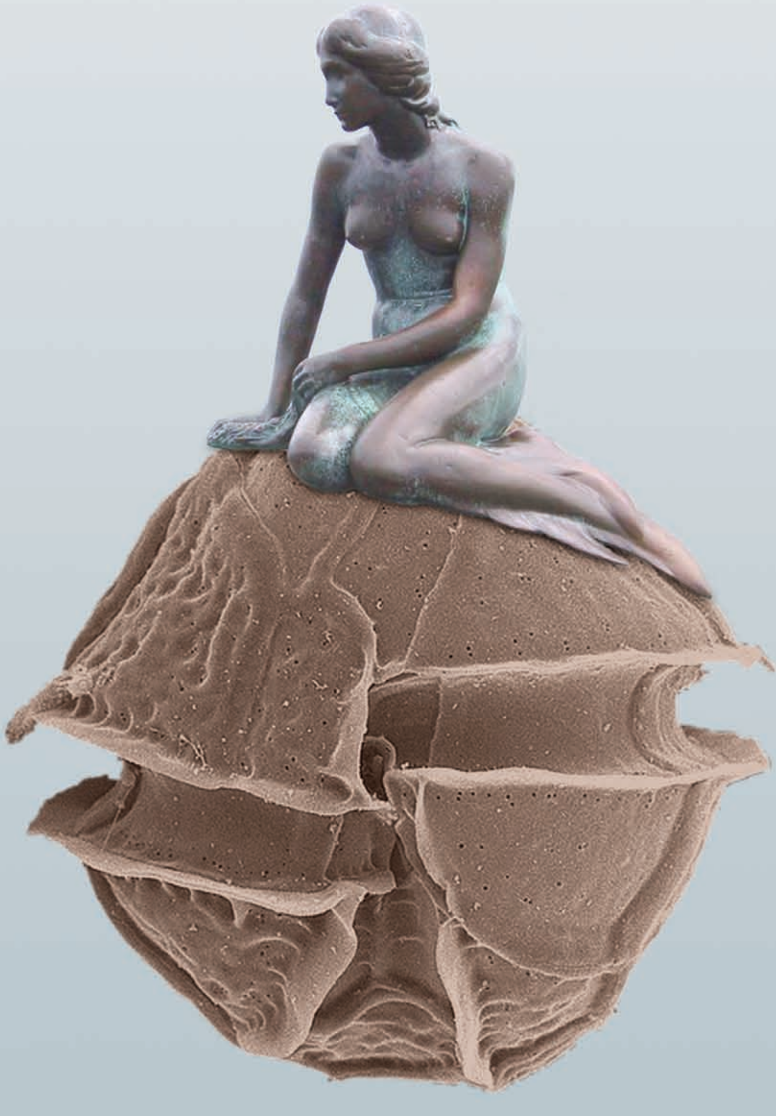
FOR THE STUDY OF HARMFUL ALGAE

12th International Conference on Harmful Algae

PROGRAMME and ABSTRACTS



Copenhagen, Denmark

4-8 September 2006

12th International Conference on Harmful Algae

PROGRAMME AND ABSTRACTS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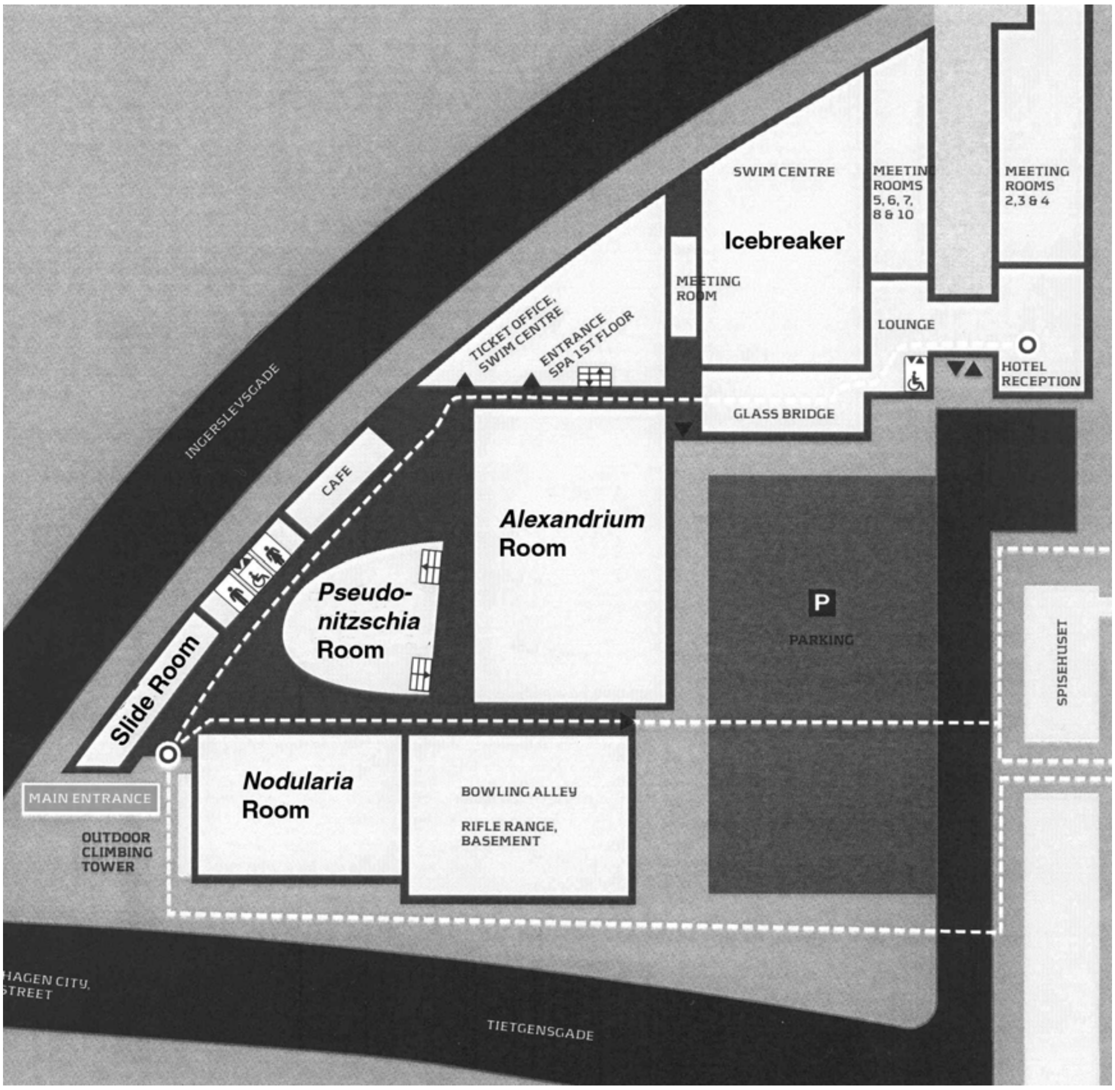
VENUE MAP



Alexandrium Room: Main lecture room and location for plenaries, ISSHA Assembly and Auction

Pseudo-nitzchia Room: Second lecture room

Nodularia Room: Poster Sessions

Slide Room: This is where you submit you Power Point files and where you can contact the Organisers.To access the Slide Room go up the stairs from the lobby area.

PROGRAMME 12TH INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

TIME	MONDAY 4 SEP	TUESDAY 5 SEP	WEDNESDAY 6 SEP	THURSDAY 7 SEP	FRIDAY 8 SEP
08.30-10.30 (Monday 08.00-10.30)	08.00-09.15 Registration Opening 9.50-10.30 Plenary I: P. Hess: What's new in toxins?	8.30-9.05 Plenary III: E. Granéli, Top-down bottom-up control of HAB dynamics 9.10-10.30 Session 5: Population dynamics 1 Session: 6: Toxicology 1	Symposia • HABs as ecosystem disrupters • Health aspects • Taxonomy-the species concept • Clay and HAB mitigation • Toxicology of toxin analogues	8.30-9.05 Plenary V: B. Dale, Anthropogenic input and climate change: effects on harmful algae 9.10-10.30 Session 13: Life cycles Session 14: Food chains	8.30-9.05 Plenary VII: V. Armbrust, Diatom genomics: new insights into diatom toxicology 9.10-10.30 Session 17: Genomics Session 18: Ecology and oceanography 1
10.30-11.00	Health break	Health break	Health break	Health break	Health Break
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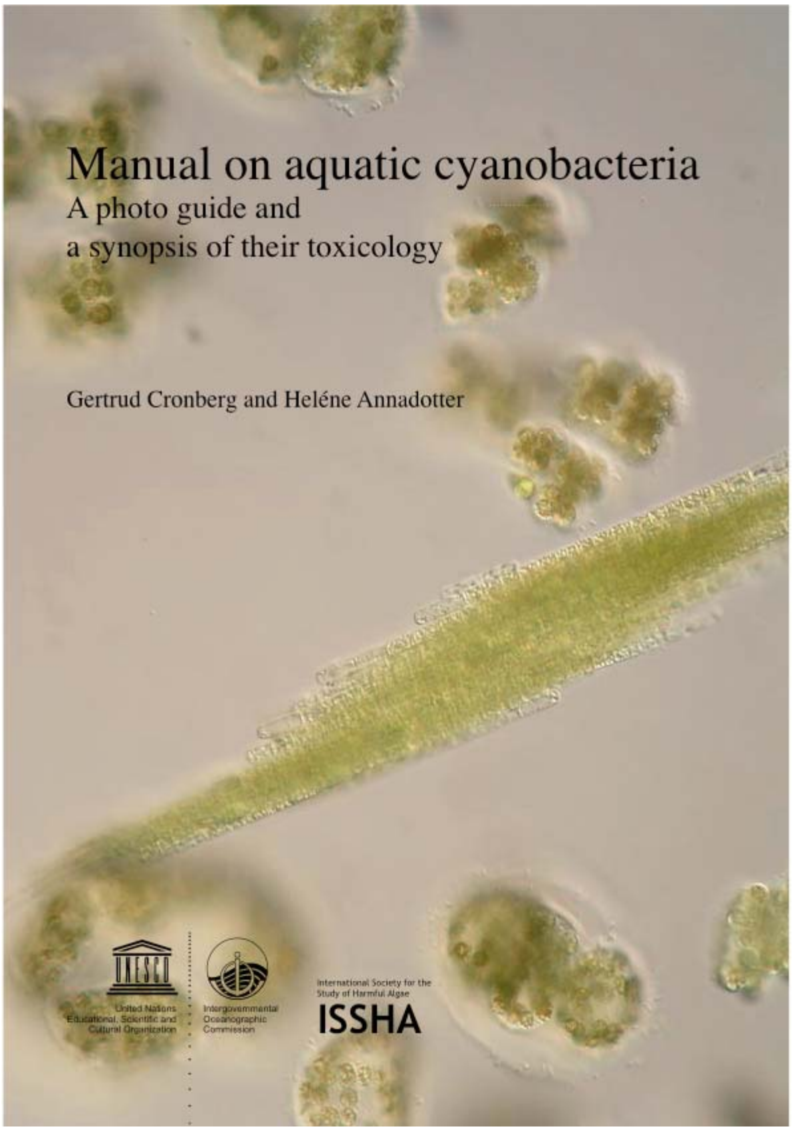
16.15-16.40	Health break	Health break	Cont.	Health break	Health Break 15.35-16.00
16.40-18.00	16.40-17.30: Poster session	Session 11: Genetics Session 12: Toxicology 2	Cont.	ISSHA Assembly, vote on 2010 venue ISSHA Auction and bar in Alexandrium Room	16.00-16.30 D. Anderson: Summing-up 16.30-16.50 M Zhou, KC Ho: HAB in China
Evening	18:00 Reception at the Copenhagen Town Hall	18.00-21.00 Poster session	MERHAB PCRWS Workshop (BI)		19.00 Dep. tour boats 19.45: Mermaid Dinner Party

NEW RELEASE:

Manual on aquatic cyanobacteria

A photo guide and a synopsis of their toxicology

Gertrud Cronberg & Heléne Annadotter
Lund University, Sweden



Potentially harmful cyanobacteria occur widespread in the aquatic environment and this manual treats their taxonomy, identification, and toxicology across freshwater, brackish and marine environments.

Published August 2006
110 pages
ISBN 87-990827-0-5
Order at: www.issha.org

ORAL PRESENTATION PROGRAMME

VERY IMPORTANT:

All oral presentations must be submitted to the secretariat (1st floor close to entrance) well in advance:

- **Presentations for the morning sessions no later than 15.00 pm the day before**
- **Presentations for the afternoon programme no later than 10.00 am the same day**

ORAL PROGRAMME

MONDAY

SESSION: PLENARY I – TOXINS PL.01
Date of session: Monday 4 Sep 2006
Start of session: 9:50:00 AM
End of session: 10:30:00 AM
Venue: **Alexandrium Room**

What's new in toxins PL.01-01
Hess
Convener: Moestrup

SESSION: TOXIN SYNTHESIS O.01
Date of session: Monday 4 Sep 2006
Start of session: 11:00:00 AM
End of session: 1:00:00 PM
Venue**: Pseudo-nitzschia Room**

Convener: Doucette

On NtcA and Fur in microcystin production in *Microcystis aeruginosa* PCC 7806, and their expression under varying nutrient regimes O.01-01
Root, Neilan

The role of transposition in cyanobacterial toxin variation and distribution: implications for toxic bloom monitoring O.01-02
Roberts, Neilan

Genetic and physiological analysis of a toxin gene cluster in two freshwater cyanobacteria, *Anabaena circinalis* and *Cylindrospermopsis raciborskii* T3 .... O.01-03
Cavaliere, Kellmann, Pomati, Neilan

Comparative study of the paralytic shellfish toxin-producing dinoflagellates, *Alexandrium tamarense* and *Gymnodinium catenatum*, by 2-DE and mass spectrometry O.01-04
Chiu, Chan, Gu

Application of saxitoxin-conjugated affinity gel for the detection of macromolecules involved in toxin dynamics in scallops O.01-05
Watanabe, Nakaji, Oshima

SESSION: PUBLIC HEALTH O.03

Date of session: Monday 4 Sep 2006
Start of session: 3:00:00 PM
End of session: 4:20:00 PM
Venue: **Pseudo-nitzschia Room**

Convener: Cembella

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Kawabata, Landsberg, Hall

Human dimensions research needs for HAB mitigation: a Washington coast example O.03-02
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A 5-day follow-up study after exposure to *Karenia brevis* toxic aerosols O.03-03
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Dalpra, Pierce, Henry, Baden

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Date of session: Monday 4 Sep 2006
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End of session: 4:20:00 PM
Venue: **Alexandrium Room**

Convener: Rengefors

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Do variations in pH and carbon levels affect the growth of potentially toxic diatoms? O.04-02
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Examination of the relevance of micropredatory dinoflagellates to ichthyotoxicity in the natural environment O.04-03
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Linking organic nutrients to HAB bloom dynamics: ELF as a tool for monitoring enzyme activities in cultures and field populations O.04-04
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End of session: 9:10:00 AM
Venue: **Alexandrium Room**

Top-down bottom-up control of HAB dynamics PL.03-01
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Convener: Hallegraeff

SESSION: POPULATION DYNAMICS 1 .... O.05
Date of session: Tuesday 5 Sep 2006
Start of session: 9:10:00 AM
End of session: 10:30:00 AM
Venue: **Alexandrium Room**

Convener: Hallegraeff

Discrimination and dynamics of naturally occurring mixed *Alexandrium* populations using rRNA targeted fluorescent oligonucleotide probes O.05-01
Touzet, Raine

Artificial neural network approaches to one-step weekly prediction of *Dinophysis acuminata* blooms in Huelva (Western Andalucia, Spain) O.05-02
Velo, Gutiérrez-Estrada

Temporal changes in microcystin-producing and non-microcystin-producing Microcystis populations of a Japanese Lake O.05-03
Yoshida

Vertical migration: an key strategy for the ecological success of the toxic dinoflagellate *Gymnodinium catenatum* in south east Tasmania, Australia O.05-04
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Start of session: 9:10:00 AM
End of session: 10:30:00 AM
Venue: **Pseudo-nitzschia Room**

Convener: Edler

Date of session: Tuesday 5 Sep 2006
Start of session: 11:00:00 AM
End of session: 1:00:00 PM
Venue: **Alexandrium Room**

Convener: Zingone

Date of session: Tuesday 5 Sep 2006
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Venue: **Alexandrium Room**

Convener: Juel Hansen

Date of session: Tuesday 5 Sep 2006
Start of session: 4:40:00 PM
End of session: 6:00:00 PM
Venue: **Alexandrium Room**

Convener: Armbrust

Date of session: Tuesday 5 Sep 2006
Start of session: 4:40:00 PM
End of session: 6:00:00 PM
Venue: **Pseudo-nitzschia Room**

Convener: Cronberg

THURSDAY

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Date of session: Thursday 7 Sep 2006
Start of session: 8:30:00 AM
End of session: 9:05:00 AM
Venue: **Alexandrium Room**

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Dale
Convener: Andersen

SESSION: LIFE CYCLES O.13
Date of session: Thursday 7 Sep 2006
Start of session: 9:10:00 AM
End of session: 10:30:00 AM
Venue: **Alexandrium Room**

Convener: Dale

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Life cycle of *Pyrodinium bahamense* var. *compressum* (Dinophyceae) in culture O.13-02
Sakamoto, Yamaguchi, Furio

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Start of session: 9:10:00 AM
End of session: 10:30:00 AM
Venue: **Pseudo-nitzschia Room**

Convener: Christoffersen

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Accumulation and transfer of the amnesic shellfish poisoning toxin domoic acid in the marine food web off the Portuguese coast O.14-02
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Grazing, prey selectivity and toxin content of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa* feeding on *Dinophysis* spp. assemblages O.14-03
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SESSION: PLENARY VI - TAXONOMY PL.06
Date of session: Thursday 7 Sep 2006
Start of session: 11:00:00 AM
End of session: 11:35:00 AM
Venue: **Pseudo-nitzschia Room**

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Convener: Larsen

SESSION: TAXONOMY O.15
Date of session: Thursday 7 Sep 2006
Start of session: 11:40:00 AM
End of session: 1:00:00 PM
Venue: **Pseudo-nitzschia Room**

Convener: Lewis

Characterization of NW Mediterranean *Karlodinium* spp. (Dinophyceae) strains using morphological, molecular, chemical and physiological methodologies O.15-01
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Date of session: Thursday 7 Sep 2006
Start of session: 11:00:00 AM
End of session: 1:00:00 PM
Venue: **Alexandrium Room**

Convener: Ho

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Start of session: 8:30:00 AM
End of session: 9:05:00 AM
Venue: **Alexandrium Room**

Diatom genomics: new insights into diatom toxicity **PL.07-01**
Armbrust
Convener: Neilan

SESSION: GENOMICS **O.17**
Date of session: Friday 8 Sep 2006
Start of session: 9:10:00 AM
End of session: 10:30:00 AM
Venue: **Pseudo-nitzschia Room**

Convener: Neilan

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Identification of cellular stress and death-associated genes in *Karenia brevis* as potential biomarkers for bloom termination **O.17-03**
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Start of session: 9:10:00 AM
End of session: 10:30:00 AM
Venue: **Alexandrium Room**

Convener: Pitcher

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Venue: **Pseudo-nitzschia Room**

Convener: Fukuyo

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Massive fish kills in the Philippines caused by *Cochlodinium* and *Prorocentrum* **O.20-03**
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End of session: 3:35:00 PM
Venue: **Alexandrium Room**

Convener: Godhe

Date of session: Friday 8 Sep 2006
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End of session: 3:35:00 PM
Venue: **Pseudo-nitzschia Room**

Convener: Tester

SESSION: SUMMING UP
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Start of session: 4:00:00 PM
End of session: 4:30:00 PM
Venue: **Alexandrium Room**

Anderson

SESSION: HAB in China
Date of session: Friday 8 Sep 2006
Start of session: 4:30:00 PM
End of session: 4:50:00 PM
Venue: **Alexandrium Room**

Zhou, Ho

ORAL ABSTRACTS

PL.01
What's new in toxins

Session: PL..01 - Plenary I - Toxins
Presentation time: 09:50 - 10:30

P Hess

Marine Institute, GALWAY, Ireland

Toxins are at the heart of the problem of harmful algal blooms and therefore many disciplines around HABs and food safety rely on chemical data to facilitate a multitude of approaches. This presentation will illustrate the role of chemistry knowledge through the review of recent developments in structure elucidation, toxicology, analysis and ecological studies. Recently discovered compounds and their relevance to food safety will be described, with particular focus on derivatives of known parent toxins. The impact of basic knowledge on the behaviour of toxins in shellfish tissues on risk assessment and management will be discussed at the example of a recent review of azaspiracids and other lipophilic toxins.
The potential of novel liquid chromatographic techniques for the fast and cost-effective analysis of shellfish toxins will be outlined at the example of ultra-high pressure liquid chromatography. Recent developments on tools for quality control, including standards and reference materials will be discussed as well as results from recent proficiency testing exercises. Finally, the usefulness of chemical analysis to field studies will be discussed at the example of the distribution of lipophilic toxins between the water column and shellfish during a toxic event in Ireland in 2005.

O.01-01
On NtcA and Fur in microcystin production in *Microcystis aeruginosa* PCC 7806, and their expression under varying nutrient regimes

Session: O.01 - Toxin synthesis
Presentation time: 11:00 - 11:20

HR Root, BA Neilan

University of New South Wales, SYDNEY, Australia

Toxic cyanobacteria present a worldwide challenge of bloom management and prediction of toxic events, involving toxins about which there is little understanding. The role and function of the hepatotoxin microcystin in cyanobacteria continues to be under debate. The mode of transcriptional regulation of microcystin, incorporating DNA binding proteins was investigated. The nitrogen and iron dependent transcription factors NtcA and Fur were identified from *M. aeruginosa* PCC 7806, over-expressed in *E. coli*, and utilised by mobility shift assay to determine their binding characteristics to the microcystin synthetase gene cluster promoter, mcyA/D.
M. aeruginosa PCC 7806 cultures were grown under varying iron and nitrogen regimes in order to observe the expression of the transcription factors NtcA and Fur over the growth cycle, utilising real-time PCR. This data was also compared to the expression of the toxin gene McyB over the same growth period, in addition to measurement of culture toxicity.

These studies suggested a role for iron and nitrogen in the transcriptional control of microcystin synthesis. By identifying the expression characteristics of NtcA and Fur under varying nutrient regimes, a greater understanding of the link between nutrient levels in the environment and microcystin production in the cyanobacteria present may be possible.

O.01-02
The role of transposition in cyanobacterial toxin variation and distribution: implications for toxic bloom monitoring

Session: O.01 - Toxin synthesis
Presentation time: 11:20 - 11:40

AA Roberts, BA Neilan

University of New South Wales, SYDNEY, Australia

Many cyanobacteria are capable of producing toxins such as microcystin and nodularin. These toxins are produced via a multi-enzyme complex which is encoded by a toxin gene cluster. Cyanobacterial toxicity is arbitrarily distributed across species and genera, as there are both toxic and non toxic strains within morphologically identical species. The identification of putative transposases downstream of every sequenced toxin gene cluster suggests the involvement of transposition in the lateral gene transfer (LGT) of toxicity between species. This is significant in terms of bloom management as presently non-toxic strains could potentially acquire toxicity via LGT. The aim of this project was to use PCR and transcriptional analysis to better understand the mechanisms of genetic rearrangements and distribution of toxicity in cyanobacteria. Investigation of regions flanking the *Microcystis aeruginosa* PCC7806 microcystin gene cluster suggested that recombination events may have occurred. However, transcriptional analysis indicated that under normal culture conditions the putative transposases associated with the microcystin and nodularin gene clusters were not transcribed. Therefore cultures were exposed to UV light, nutrient stress and heat shock to determine conditions which induced transcription of the transposases and thus may have led to toxin gene cluster mobilisation.

O.01-03
Genetic and physiological analysis of a toxin gene cluster in two freshwater cyanobacteria, *Anabaena circinalis* and *Cylindrospermopsis raciborskii* T3

Session: O.01 - Toxin synthesis
Presentation time: 11:40 - 12:00

Rosalia Cavaliere, Ralf Kellmann, Francesco Pomati, Brett Neilan

University of Sydney, SYDNEY, Australia

Blooms of cyanobacteria are a common feature of many fresh and marine water bodies world-wide, and have been observed throughout history. Infestation of cyanobacteria in sediment which are rich in calcium and phosphorus has been reported. Cyanobacterial blooms are often characterised by the presence of various toxins. Saxitoxin and its analogues (paralytic shellfish toxins) are one of these groups, recognized as a major health risk. Toxicity is highly variable and unpredictable and may affect water bodies that are used as a source of drinking water and for

recreational purposes. In view of this there is a need to understand the genetics and physiology that controls the production of these toxins.
Recently the molecular genetic basis for the production of PSP-toxins has been elucidated. The aims of this work were the characterisation of the transcript organisation of the PSP-toxin cluster in the cyanobacteria *Anabaena circinalis* and *Cylindrospermopsis raciborskii* and to study the effects of phosphorus depletion on PSP-toxin gene expression and toxin production. Depletion of phosphorus in the media has been shown to increase the toxin production in dinoflagellates and cyanobacteria. The PSP-toxin cluster operon is bidirectionally transcribed from a central promoter region containing several regulatory motifs.

O.01-04
Comparative study of the paralytic shellfish toxin-producing dinoflagellates, *Alexandrium tamarense* and *Gymnodinium catenatum,* by 2-DE and mass spectrometry

Session: O.01 - Toxin synthesis
Presentation time: 12:00 - 12:20

Ellen Chiu[1], L Chan[2], JD Gu[2]
HONG KONG, Hongkong
[2]The University of Hong Kong, POKULAM, Hongkong

Several genera of dinoflagellates, including *Alexandrium*, *Gymnodinium* and *Pyrodinium*, are known to produce paralytic shellfish poisoning toxins (PST). However, chemical intermediates and specific enzymes have not been identified and the full biosynthetic pathway for PST remains unresolved. In this study, the proteomes of toxic and non-toxic strains of *Alexandrium tamarense* were compared by two-dimensional gel electrophoresis (2-DE). Differentially expressed proteins found in the toxic strain but not in the non-toxic strain are thought to be related to PST production. Further validations of these proteins involved:
(1) comparison with another phylogenetically distant toxic species, *Gymnodinium catenatum*, to eliminate those proteins not related to toxin production; and (2) determination of internal amino acid sequences by MALDI TOF-MS/MS spectra of the tryptic peptides of proteins of interest. Results showed approximately 400 common proteins spots found in both toxic and non-toxic strains of *Alexandrium tamarense*, and 150 protein spots were unique to each strain. Preliminary results showed the protein profile of *Alexandrium tamarense* was more complex than *Gymnodinium catenatum*. However, some common proteins were identified between the toxic strain of *Alexandrium tamarense* and *Gymnodinium catenatum*, which are currently investigated to elucidate toxin biosynthesis. This study suggests proteomics is a robust technique in protein differentiation.

O.01-05
Application of saxitoxin-conjugated affinity gel for the detection of macromolecules involved in toxin dynamics in scallops

Session: O.01 - Toxin synthesis
Presentation time: 12:20 - 12:40

R Watanabe, K Nakaji, Y Oshima
Tohoku University, SENDAI, Japan

It is highly possible that macromolecules are involved in the transportation and the accumulation of saxitoxins in marine organisms. As an efficient tool to detect the macromolecules having affinity to saxitoxins, we developed saxitoxin-conjugated affinity gel. In this paper, we report the 1st trial to test its efficiency using the extract of scallop *Patinopecten yessoensis*. The mantle was chosen as the target organ because it retained the toxins much longer than other organs.
The affinity gel and a control acetylated gel without toxin-ligand were prepared according to the previously reported procedures. The extracts of toxic and non-toxic scallop samples were fractionated with two types of gel in spin-columns and the retained proteins were compared by SDS-PAGE. Several specific bands detected in the samples were analyzed with peptide-mass fingerprinting method and identified by the search engine MS-Fit based on NBCInr. database. So far, tropomyosins were found as candidate proteins. The actual toxin binding properties and the role in toxin accumulation of them are now under investigation. Since the procedure is simple and requires only short period, it will be an effective tool as the first screening for saxitoxin-binding proteins.

O.01-06
Amphidinols and karlotoxins – brothers in arms part deux: structural similarities

Session: O.01 - Toxin synthesis
Presentation time: 12:40 - 13:00

AR Place[1], JE Adolf[1], TR Bachvaroff[1], JE Peng[2], MT Hamann[2]

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Amphidinols have common structural features characterized by 2 ether rings, 7 double bonds involving a conjugated triene and an exomethylene, a branching methyl, an olefinic methyl, and polyhydroxyl groups. We recently finished the planar structure of karlotoxin-2 (KmTx2) which was found to have a molecular formula of C67H121ClO24. KmTx2 contained 2 ether rings, 5 double bonds involving a conjugated diene and exomethylene, a branching methyl, and polyhydroxyl groups. No olefinic methyl group was found. A sulphated derivative of KmTx2 (in the hydrophilic segment) was also isolated and found to be significantly less toxic. The determination of the relative and absolute configuration of the polyhydroxyl linear molecular of KmTx2 remains to be completed and will be reported in due course. Given these extensive structural similarities the biological activities of amphidinols and karlotoxins are also remarkably similar. Both toxins show sterol dependent antifungal, cytotoxic and hemolytic activities in the submicromolar range. Karlotoxins are also ichthyotoxic at concentrations less than 0.2 µg/ml. Based on experimental data gathered from *Karlodinium* we hypothesize that both groups of toxins have evolved to assist in predator avoidance and prey capture for mixotrophic growth.

O.02-01
Utilization of DOM by *Prymnesium parvum* from anthropogenic sources, detected by stable isotopes, and effects on cell toxicity

Session: O.02 - Ecophysiology 1
Presentation time: 11:00 - 11:20

E Lindehoff, E. Granéli
University of Kalmar, KALMAR, Sweden

The ichthyotoxic haptophyte *Prymnesium parvum* is a known phagotroph, however its osmotrophic behaviour is practically unknown. After cultivating *P. parvum* cells under nitrogen and phosphorus sufficient and deficient conditions concentrates of high molecular weight (>1kDa) dissolved organic matter (DOM) containing 20µM of dissolved organic nitrogen (DON) were added to the cultures. We used DOM concentrate from riverine, rainwater and sewage effluent (P removed chemically). Cell toxicity was measured and preliminary results showed that toxicity decreased when DOM was added to nitrogen and phosphorus deficient cultures. Delta 15-N analysis showed an uptake of DON from all DOM sources by nitrogen deficient *P. parvum* cells with decreased values when rainwater DOM was provided and increased values with additions of DOM from sewage and riverine waters. Uptake of carbon from rainwater and sewage DOM concentrates was traced in the nitrogen deficient *P. parvum* cells by decreasing delta 13-C values. In this experiment we show that *P. parvum* is able to utilize DON from three tested anthropogenic sources and that the assimilation rate and effect on toxicity is dependent on the nutrient status of the cell.

O.02-02
Slimy threads, sneaky feeding, and other secrets of an invasive raphidophyte

Session: O.02 - Ecophysiology 1
Presentation time: 11:20 - 11:40

K Rengefors[1], C Pålsson[1], RI Figueroa[2], L Hansson[1]
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During the past decades, the invasive and bloom-forming raphidophyte *Gonyostomum semen* has been spreading to forest lakes throughout Scandinavia. The blooms have increased both in frequency and magnitude, and cause skin irritation to bathers as well as clogging of pipes. However, very little is know about *Gonyostomum*'s ecology and why it dominates in humic lakes. Suggested explanations have been its ability to migrate vertically, resting cysts, ability to utilize humic substances, or resistance to grazing. We have investigated its life cycle, as well as growth with prey and humic substances . We found that *Gonyostomum* can produce both temporary cysts as well as sexually produced resting cysts. Growth experiments showed that both cell density and growth rate were highest when *Gonyostomum* was grown together with the small alga *Rhodomonas*. Moreover, cells grown with humic acids grew better than controls, and growth rates were highest in treatments with highest humic acid content. Ingestion rate experiments showed that *Rhodomonas* disappeared when exposed to *Gonyostomum*. However, we found no evidence of ingestion. We suggest that *Gonyostomum* is mixotrophic, and that this ability together with grazer resistance, may in part explain its competitive advantage.

O.02-03
Growth and survival of *Dinophysis acuminata*, a phototrophic marine dinoflagellate causing diarrhetic shellfish poisoning

Session: O.02 - Ecophysiology 1
Presentation time: 11:40 - 12:00

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[3]Chonnam National University, GWANGJU, South Korea

Many scientists have attempted to cultivate dinoflagellate *Dinophysis* species using various culture media and potential prey organisms without any reported success. To investigate the growth of *Dinophysis* species, we isolated *D. acuminata* cells from seawater samples collected at Masan Bay, Korea and incubated the isolate under growth conditions (60 µmol m^{-2} s^{-1}, L/D cycle of 14:10) supplying potential prey species including *Teleaulax* sp., a cryptophyte. Further, to know the effect of starvation on growth of *D. acuminata*, we incubated the culture in the absence of prey organisms under the light:dark cycle as well as continuous dark condition, and monitored cell abundance of *D. acuminata* over times. In this study, quantitative importance of potential prey organism for the growth and survival of *D. acuminata* will be discussed.

O.02-04
Broad predator-prey relationship among the mixotrophic red-tide dinoflagellates

Session: O.02 - Ecophysiology 1
Presentation time: 12:00 - 12:20

HJ Jeong[1], YD Yoo[1], NS Kang[1], JY Song[2], TH Kim[2], WH Yih[2], JY Park[1]
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[2]Kunsan National University, KUNSAN, South Korea

We investigated predator-prey relationship among phototrophic red-tide dinoflagellates. There are broad predator and prey relationships among red-tide dinoflagellates (*Akashiwo sanguinea*, *Alexandrium tamarense*, *Amphidinium carterae*, *Gymnodinium catenatum*, *G. impudicum*, *Heterocapsa triquetra*, *Lingulodinium polyedrum*, *Prorocentrum donghaiense*, *P. micans*, *P. minimum*, *P. triestinum*, and *Scrippsiella trochoidea*). *Akashiwo sanguinea* and *L. polyedrum* were able to ingest the small red-tide dinoflagellates *P. minimum*, *H. triquetra*, *S. trochoidea*, and *A. tamarense*. Maximum specific growth rates of *L. polyedrum* on *P. minimum* and *S. trochoidea* were 0.254 and 0.303 d^{-1}, respectively, under a 14:10 h light-dark cycle of 50 µmol m^{-2} s^{-1}, while their growth rates without added prey were 0.157 and 0.182 d^{-1}, respectively. Maximum ingestion rates of *L. polyedrum* on *S. trochoidea* and *P. minimum* were 0.20-0.36 ngC $grazer^{-1}$ d^{-1}. The calculated grazing coefficients of *L. polyedrum* on small *Prorocentrum* spp. and *S. trochoidea* were up to 0.026 and 0.011 h^{-1}, respectively. The results of the present study suggest that *L. polyedrum* sometimes have a potentially

considerable grazing impact on populations of small *Prorocentrum* spp. and *S. trochoidea*. The feeding by larger red-tide dinoflagellates on smaller red-tide dinoflagellates may be a driving force for succession of dominant species during serial red tides.

O.02-05
Distribution and ecology of toxic and non-toxic strains of *Microcystis* populations in North American lakes

Session: O.02 - Ecophysiology 1
Presentation time: 12:20 - 12:40

CJ Gobler[1], DL Berry[1], TW Davis[1], GL Boyer[2], SW Wilhelm[3]

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Harmful algal blooms often comprises toxic and non-toxic strains of the same phytoplankton species, which cannot be differentiated microscopically. The identification of the operon responsible for the synthesis of microcystin (microcystin synthetase genes, mcyA-E) has allowed for the quantification of toxic subpopulations of cyanobacteria within mixed field populations. We quantified the densities of toxic and non-toxic *Microcystis* populations in western Lake Erie, USA, during the summer of 2005 using the 16S and mcyD genes in a quantitative PCR format. While total *Microcystis* cell densities ranged from 10^3 to 10^5 cells/ml, toxic cells with the mcyD gene were typically only fraction of this population (1 – 55%). The highest levels of microcystin (0.5µg/L) were found in regions with the highest densities of toxic cells, suggesting sub-population dynamics may influence ecosystem toxicity. Experimental incubations indicated that inorganic nutrient enrichment (both nitrate and phosphate) was capable of significantly enhancing total algal biomass and non-toxic *Microcystis* cell densities but did not significantly alter toxic cell densities, suggesting inorganic nitrogen and phosphorus may not directly stimulate bloom toxicity. The effects of environmental factors on the dynamics of toxic and non-toxic strains of *Microcystis* from other North American lakes will also be presented.

O.02-06
Dinoflagellate and raphidophyte blooms and the 15°C barrier

Session: O.02 - Ecophysiology 1
Presentation time: 12:40 - 13:00

TJ Smayda

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Experimental data on the temperature-cell division relationships are analyzed for ca. 30 dinoflagellate and raphidophyte species representative of various toxicity modes, bloom patterns and Life-form Types recognized by Smayda and Reynolds (2001). A 15 °C barrier to bloom development of major, harmful flagellates, with two distinct groups separated by this 'bloom threshold' temperature, is evident. Temperatures below or near 15 °C suppress cellular growth of the raphidophytes and almost all toxic dinoflagellates examined, their optimal growth temperatures usually • 20 °C. Dinoflagellates inhibited at • 15 °C cluster in Life-form Types IV,V, VI. Dinoflagellate species capable of cell division below 15 °C, and which often bloom close to this temperature, are primarily Life-form Types I, II, III. Such 'cold water tolerant' species tend to be non-toxic or, if toxic, ichthyotoxic. *Alexandrium tamarense* is a conspicuous transitional species which bridges the 15 °C barrier. Some ecological consequences of the 15 °C barrier and the effect of temperature on motility are considered, including the corollary that temperature, not biophysics or ecology, generally constrains flagellate blooms to periods when stratified waters prevail.

PL.02

The public policy of harmful algal blooms

Session: PL..02 - Plenary II
Presentation time: 14:15 - 14:50

Porter Hoagland

Woods Hole Oceanographic Institution, WOODS HOLE, United States of America

Harmful algal blooms (HABs) are natural hazards that have been recognized as threats to human health and welfare worldwide. Arguments have been made in the scientific literature that HABs have become more widespread, even as human coastal populations continue to expand, suggesting that the potential for adverse impacts is intensifying. In the face of such threats, national and local governments must make decisions about potential policy responses. These decisions must be undertaken subject to significant budget constraints and in the context of the need to respond to threats from other types of natural and human hazards. Economic analysis may be useful in assisting decision-makers in their choice of the most appropriate policy response to any specific HAB event. All too often, however, a loose accounting of potential financial losses due to HABs is used inappropriately to argue for the need for specific policy responses. I discuss the problems with these approaches, and outline methods for compiling and undertaking analyses of the data that can be helpful in making rational decisions about policy responses to HABs. I conclude with the observation that there is a need for a closer collaboration between HABs scientists and marine resource economists in this field.

O.03-01

Dietary uptake of dinoflagellate-derived saxitoxins in non-toxic puffer-fish: implications for global seafood safety

Session: O.03 - Public Health
Presentation time: 14:55 - 15:15

JR Deeds[1], SM Etheridge[1], C Gieseker[1], CS Cheely[1], R Reimschuessel[1], JP Abbott[2], K Kawabata[2], JH Landsberg[2], S Hall[1]

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Between 2002-2004, 28 fish poisoning cases were caused by the consumption of southern puffer-fish (*Sphoeroides nephelus*), from the Indian River Lagoon (IRL), Florida, USA. Saxitoxin, not tetrodotoxin, was reported from unconsumed filets after the first cases. *Pyrodinium bahamense* is now considered the putative toxin source. In contrast to reports for tetrodotoxic fish, southern puffer muscle contained high levels of toxin (up to 5000 µg STX/100 g) with little to no toxin in liver. We have shown that non-toxic northern puffer-fish (*S. maculatus*) accumulate saxitoxins from naturally and artificially contaminated feed in the same tissue distribution pattern as found in toxic IRL southern puffer-fish. We have further demonstrated that northern puffer-fish accumulate dietary tetrodotoxin in skin epidermal sacciform cells as described for naturally tetrodotoxic *Tetraodon* sp. The toxicity of certain puffer-fish species has been well known for thousands of years. Globally, deaths continue to occur due to the consumption of various puffer species, even in Japan where strict regulations exist. But not all puffer species are toxic. In the US, northern puffer-fish were harvested commercially for decades. Several puffer-fish species possess the ability to accumulate dietary saxitoxin as well as tetrodotoxin, which has implications for seafood safety world-wide.

O.03-02

Human dimensions research needs for HAB mitigation: a Washington coast example

Session: O.03 - Public Health
Presentation time: 15:15 - 15:55

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Human impacts of HABs include illness and mortality, lost revenue and jobs, and disruption of subsistence and cultural practices. Preventing and mitigating impacts requires human dimensions (HD) research, e.g., to guide risk communication, identify susceptible populations, assess socioeconomic impacts, improve disease surveillance, and coordinate agencies and stakeholders. A new report based on the US National HAB plan and led by NOAA's National Centers for Coastal Ocean Science, Harmful Algal Research and Response: a Human Dimensions Strategy (HARR-HD), charts a course to achieve these and other priorities. We describe the interdisciplinary and multi-agency process for developing HARR-HD, illustrate HD research needs through case stud(ies), and set the stage for open discussion of HD efforts and lessons learned across agencies/nations. A case study will focus on the US Pacific Coast. Governmental closures, necessary to protect public health, can cause an annual loss of up to 400,000 razor clam-digging trips, effectively disrupting the recreational traditions of tens of thousands, diminishing the collective identity of communities, causing roughly $10 million in lost annual income, and degrading the fragile trust between regulated and regulating communities. Washington Department of Fish and Wildlife is a key partner in developing HARR-HD.

O.03-03

A 5-day follow-up study after exposure to *Karenia brevis* toxic aerosols

Session: O.03 - Public Health
Presentation time: 15:55 - 16:15

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Blooms of the toxic dinoflagellate, *Karenia brevis*, occur annually around the Gulf of Mexico. A unique feature of this organism is the incorporation of the toxins into the marine aerosol. Animals, including humans, then inhale the toxins which cause respiratory irritation. Recent studies have demonstrated acute changes in both symptoms and spirometry in asthmatics after a 1-h exposure to these toxic aerosols. This study investigated if there were latent or sustained effects after the initial beach exposure during and not during a documented Florida red tide. Asthmatics who participated in the 1-h exposure study were asked to keep a symptom diary and to measure their peak flow daily for 5 days after exposure. Environmental air samplers were placed on an inland transect line to document continuing toxic marine aerosols. Although there was no statistical change in the peak flow measurements over the 5 days, when the number of symptoms were scored, a significant increase in symptoms occurred over the 5-day exposure period compared to the non exposure period. These findings suggest that asthmatics exposed to *K. brevis* aerosols may continue to have symptoms lasting long after their initial beach exposure to the Florida red tide toxin aerosols.

O.04-01
Exopolymeric secretions in HABs: how flow, diffusion and bioengineering depends on lengthscale

Session: O.04 - Ecophysiology 2
Presentation time: 14:55 - 15:15

IR Jenkinson[1], T Wyatt[2]
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We all have intimate experience of exopolymeric secretions (EPSs), and other mixtures of biomaterials, like faeces or food. Such materials are neither solids nor liquids, and their study is called Rheology. Some HABs use EPSs to gel the water and engineer their environment at lengthscales (LSs) £1µm to ~1m, trapping bubbles, producing 'jelly' colonies, etc. Marine snow (LSs ~1mm) and Adriatic aggregates (LS ~1m) are also gels. The viscosity η of such structures is many times higher than that of pure seawater at the shear rates concerned, but it changes with shear rate, γ. η is (usually) positively related to chlorophyll a concentration (chl), confirming its algal origin. Homeowners know that for a given head of water and concentration of the biomixture, sewage, narrow sewer pipes clog more readily than wide ones. Yet only recently have general models been validated, that incorporate LS in flows of heterogeneous materials. In sewage sludge flowing in tubes, η gets bigger with smaller tube diameter (and smaller γ). We will present the basis of rheology, and also new insights into how to treat rheology as a function of lengthscale [f(LS)] in heterogeneous materials. Next we will show how f(LS) can help show how bioengineering affects flow and diffusion in HABs, particularly at small scales.

O.04-02
Do variations in pH and carbon levels affect the growth of potentially toxic diatoms?

Session: O.04 - Ecophysiology 2
Presentation time: 15:15 - 15:35

Nina Lundholm[1], Yuichi Kotaki[2]
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PH level and concentration of inorganic carbon has been found to vary in coastal marine areas. The effects of these variations on coastal diatoms are poorly known. We hence examined the effects of variations in inorganic carbon concentrations and pH on costal bloom forming diatoms like the potentially toxic species *Pseudo-nitzschia multiseries* and *Nitzschia navis-varingica* at conditions where nutrients were not limiting growth. The diatoms were studied using semicontinous experimental set-ups at stable pH and carbon levels or as batch culture experiments where pH and carbon was allowed to drift. For all diatoms, the main factor limiting growth was found to be pH, caused by levels of pH that one can expect to find in the field. For *N. navis-varingica* our studies did not support an effect of inorganic carbon concentrations on growth. For *P. multiseries*, however, inorganic carbon may be limiting growth in productive brackish waters, where pH levels are high and carbon concentrations low.

O.04-03
Examination of the relevance of micropredatory dinoflagellates to ichthyotoxicity in the natural environment

Session: O.04 - Ecophysiology 2
Presentation time: 15:35 - 15:55

VJ Lovko, WK Vogelbein
Virginia Institute of Marine Science, GLOUCESTER POINT, United States of America

Species of the dinoflagellate family Pfiesteriaceae have been demonstrated to feed myzocytotically on the tissues of live fish, resulting in severe epidermal erosion and death. This process of micropredation has been identified as the primary causative mechanism of fish mortality in laboratory bioassays with *Pseudopfiesteria shumwayae* and *Pfiesteria piscicida*, species previously thought to produce a potent ichthyotoxin. However, the relevance of this behaviour in the natural environment has not been examined. Here, we describe the results of studies aimed at determining the role, if any, that micropredatory dinoflagellates have in ichthyocidal activity in the natural environment. It has been suggested that loss of or damage to the epidermis of fish results in increased risk of exposure to other pathogenic agents, including toxins and secondary microbial infections. To examine this potential relationship, we exposed fish to a highly micropredatory strain of *Pseudofiesteria shumwayae* (CCMP 2089) followed by subsequent exposure to various algal toxins (including whole-cell *Karlodinium veneficum* cultures, *K. veneficum* lysate, and purified or partially purified phycotoxins, including saxitoxin, domoic acid and brevetoxin) or microbial pathogens (*Vibrio* sp., *Aphanomyces invadans*). Preliminary results indicate that sub-lethal exposure to micropredatory dinoflagellates can significantly increase susceptibility to secondary pathogenic agents.

O.04-04
Linking organic nutrients to HAB bloom dynamics: ELF as a tool for monitoring enzyme activities in cultures and field populations.

Session: O.04 - Ecophysiology 2
Presentation time: 15:55 - 16:15

ST Haley, ED Orchard, A Strojsova, ST Dyhrman

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The dynamics of harmful algal blooms (HAB) can be dramatically influenced by nutrient supply, and it is widely accepted that both the quantity and quality of the nutrient pool can impact HAB formation, persistence, and decline. Dissolved organic forms of nitrogen and phosphorus comprise a nutrient pool that is often not readily bioavailable to phytoplankton without enzymatic hydrolysis to inorganic forms. One way of assessing nutrient availability, and perhaps enabling greater understanding of the cues which allow HAB to form, is to use cell-specific enzyme assays to monitor cellular physiology. Recent advances, such as enzyme labeled fluorescence (ELF), now allow us to specifically monitor the enzyme activities associated with harmful species in complex communities. We have screened a series of cultures and field samples for alkaline phosphatase and N-acetylglucosaminidase activity using ELF to assess the presence of these activities and the extent to which they are nutrient-regulated. In the case of the dinoflagellate *Karenia brevis*, greater than 80% of the cells in samples from the Florida coast had alkaline phosphatase activity. These data and our ongoing sampling underscore the importance of the organic nutrient pool to HAB dynamics.

PL.03
Top-down bottom-up control of HAB dynamics

Session: PL..03 - Plenary III
Presentation time: 08:30 - 09:05

Edna Granéli

Kalmar University, KALMAR, Sweden

The systematic overexploitation of marine resources through fishing has led to changes at the top of the food chains. These changes have cascaded down the food chains. From lakes it has been known since around 1980 that a reduction in the number and size of larger, piscivorous (predators on smaller fishes) diminish, leads to an increase in the biomass of smaller, planktivorous fishes. This, in turn, leads to a higher predation pressure on larger zooplankton and as a consequence the grazing pressure on phytoplankton will decrease. This mechanism is especially strong in nutrient-rich waters. Thus, over-fishing and eutrophication may work synergistically to increase phytoplankton biomass. HAB species, in addition, have the advantage over other phytoplankton groups in being -to a variable extent - grazing-resistant by being unpalatable (from the production of toxins and/ or through body shape/size). Diminished grazing in combination with increasing amounts of nutrients may thus select for HAB species. I argue that changes caused by overfishing can diminish grazing, directly affecting those HAB species positively that are not totally grazer resistant, while increased nutrient availability (through eutrophication) will divert nutrients from grazing-sensitive to grazer- resistant (HAB) phytoplankton species.

O.05-01
Discrimination and dynamics of naturally occurring mixed *Alexandrium* populations using rRNA targeted fluorescent oligonucleotide probes

Session: O.05 - Population dynamics 1
Presentation time: 09:10 - 09:30

N Touzet, R Raine

National University of Ireland Galway, GALWAY, Ireland

Field investigations in Cork Harbour, Ireland have revealed a mixed population of *Alexandrium* comprising a non-toxic form of A. tamarense (West European ribotype) and a PSP toxin-producing *A. minutum* (Global Clade). The latter species is now confirmed as the causative organism responsible for historical occurrences of PSP contamination of shellfish in the area. The morphological similarity between these species has made comparative dynamics studies difficult when using conventional methodologies. This problem is compounded by the requirement to understand the processes involved in lifecycle transitions in order to determine the mechanisms that govern the initiation and termination phases of blooms. Here we report the use of LSU rRNA targeted taxa-specific fluorescent oligonucleotide probes for studies on comparative bloom dynamics of these two species within the same estuary. In addition, particular emphasis is given to the potential of FISH probes as a tool for the differentiation between vegetative cells and advanced stage planozygotes. Morphological observations carried out with both culture and field material are reported.

O.05-02
Artificial neural network approaches to one-step weekly prediction of *Dinophysis acuminata* blooms in Huelva (Western Andalucia, Spain)

Session: O.05 - Population dynamics 1
Presentation time: 09:30 - 09:50

L Velo[1], JC Gutiérrez-Estrada[2]

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[2]Universidad de Huelva, HUELVA, Spain

The Atlantic coasts of Andalucia are affected by chronic spring-summer (March to June) Diarrhetic Shellfish Poisoning (DSP) outbreaks associated with proliferations of *Dinophysis acuminata* Claparède and Lachmann. Artificial Neural Networks (ANN) have been successfully used to model primary production and has recently been tested for the prediction of harmful algae blooms. In this study, we evaluated the performance of feed forward ANN models trained to predict *Dinophysis acuminata* proliferations. ANN models were trained and tested using weekly data (five previous weeks) of *D. acuminata* cell counts from 8 stations of the Andalusian HAB monitoring programme in the coasts of Huelva between 1998 and 2004. Principal Component Analyses (PCA) were previously carried out to find out possible similarities within time series from each zone with the aim to reduce the number of areas

to model, and further the number of sampling stations. Our results show that ANN models, with a low number of input variables, are able to reproduce trends in *D. acuminata* population dynamics.

O.05-03
Temporal changes in microcystin-producing and non-microcystin-producing *Microcystis* populations of a Japanese Lake

Session: O.05 - Population dynamics 1
Presentation time: 09:50 - 10:10

Mitsuhiro Yoshida

Fukui Prefectural University, OBAMA, Japan

Temporal changes in hepatotoxin microcystin-producing and non-microcystin-producing *Microcystis aeruginosa* populations were examined in Lake Mikata, Japan. To monitor densities of the total M. aeruginosa population and potentially microcystin-producing cells, we used quantitative real-time PCR assays targeting the phycocyanin intergenic spacer (PC-IGS) and the microcystin synthetase gene (mcyA), respectively. During the sampling period, the ratios of cell numbers of mcyA genotypes to the total *M. aeruginosa* varied considerably with the range from 0.005 to 0.35. When surface nitrate concentrations increased dramatically, there was an apparent rise in cell number ratios of mcyA genotypes. As an alternative approach, the 16S-23S rDNA internal transcribed spacer (16S-23S ITS) genotyping was employed for the identification of potentially microcystin-producing and non-microcystin-producing genotypes in natural communities. The phylogenetically closely related but distinct microcystin genotypes were found between different samples when ratios of cell numbers of mcy genotypes were relatively high. Thus, our data suggest that multiple ecotypes, which are adapted to ecological parameters, might coexist within the *M. aeruginosa* community.

O.05-04
Vertical migration: an key strategy for the ecological success of the toxic dinoflagellate *Gymnodinium catenatum* in south east Tasmania, Australia

Session: O.05 - Population dynamics 1
Presentation time: 10:10 - 10:30

SIE Blackburn[1], K Wild-Allen[1], MA Doblin[2], PA Armstrong[3], CJ Bolch[4], PA Thompson[1], GM Hallegraeff[4]

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The toxic dinoflagellate *Gymnodinium catenatum* has formed recurrent blooms in the Huon Estuary, south east Tasmania since the mid 1980s. The estuary is characterised by humic-laden surface river flow and micro-tidal salt wedge estuarine circulation. A distinctive feature of the blooms is diurnal vertical migration (DVM), up to 20 metres of the water column. Laboratory studies using vertically stratified columns demonstrate that DVM facilitates nutrient retrieval at depth. In addition, *G. catenatum* grows equally well on nitrate, ammonium or urea and, in the field, *G. catenatum* utilises whichever of these nitrogen sources are available during DVM. This indicates an advantage for *G. catenatum* in its ability to access a continuous supply of nitrogen through vertical migration. A 3D-coupled hydrodynamic-ecological modelling study demonstrates that the interaction of DVM with the estuarine circulation provides a physical mechanism for the retention and accumulation of the species in the estuary. These combined laboratory, field and modelling approaches demonstrate that vertical migration is a key strategy for the ecological success of G. catenatum in south east Tasmania.

O.06-01
Early life exposure to domoic acid leads to 'silent' neurological manifestaions in juveniles and adults

Session: O.06 - Toxicology 1
Presentation time: 09:10 - 09:30

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Domoic acid is a rigid analog of the excitatory amino acid glutamate produced by the diatom genus *Pseudo-nitzschia*, and a well-characterized neurotoxin in adult animals. However, the impact of domoic acid exposure early in development is by comparison poorly understood. Toxicity occurring during development often takes on a different set of parameters, because effects on developmental processes can cause 'silent' changes that result in increased susceptibility to neurological disorders later in the life span. We will present recent findings how in utero domoic acid exposure in rats leads to 'silent' neurologic manifestations in juvenile and adult offspring. These manifestations take the form of altered performance in learning and memory tasks as well as diminished cognitive reserve as evident in response to drugs. In parallel, we will present a model that uses zebrafish (*Danio rerio*) as an alternative species to complement our developmental studies in rat. Zebrafish embryos microinjected with domoic into the yolk sac display typical domoic acid symptoms of seizures and stereotypic behaviors in rats. The zebrafish model is now being used to characterize susceptibility of domoic acid-exposed embryos to seizure-causing drugs later in life to evaluate how domoic may cause 'silent' effects that predispose adults to neurological disorders.

O.06-02
The fate of dissolved domoic and okadaic acid in presence of bacteria, copepod faecal pellets and mussel faecal matter

Session: O.06 - Toxicology 1
Presentation time: 09:30 - 09:50

JA Hagström, E Granéli
University of Kalmar, KALMAR, Sweden

In this work, we examined some of the biotic factors that could be involved in degradation of the toxins domoic acid (DA) and okadaic acid (OA) during decay of toxic algal cells. Toxin standard in surface seawater was incubated during 69 days with: 1) natural bacterial abundance from a known bloom site of toxigenic *Pseudo-nitzschia* spp. or *Dinophysis* spp., 2) 4-fold concentration of natural bacterial abundance, 3) copepod faecal pellets and 4) mussel faeces and pseudo-faeces. Degradation rates of dissolved DA in the two bacterial treatments were 2.2% and 3.6% day^{-1}, respectively. Copepod faecal pellets had no effect on DA whereas degradation was as high as 50% day^{-1} in the mussel faecal matter treatment. Degradation of OA was only found with 4-fold bacteria abundance and at low rate (0.9% day^{-1}). Even after 69 days exposition to copepod and mussels faecal matter did not result in OA degradation. Therefore, OA is a much more resistant toxin than DA. As the data suggests that some bacteria and digestive enzymes aggregated within mussel faecal matter shorten the degradation time of DA, degradation of OA in presence of other shellfish species faecal matter should be studied.

O.06-03
Ostreopsis siamensis and palytoxin-related compounds in New Zealand: a risk to human health?

Session: O.06 - Toxicology 1
Presentation time: 09:50 - 10:10

LL Rhodes[1], R Munday[2], LR Briggs[2], PT Holland[1], CO Miles[2], J Loader[2], D Jensen[2], J Cooney[2]

[1]Cawthron, NELSON, New Zealand
[2]AgResearch, HAMILTON, New Zealand

Ostreopsis siamensis occurs in New Zealand's sub-tropical waters and produces potent novel toxins with some characteristics of palytoxin. Summer blooms of *O. siamensis* have been linked to massive seaweed diebacks and to sea urchin (Evechinus chloroticus) mortalities in the far north of New Zealand over the last few years. Optimisation of growth of *O. siamensis* in vitro, using 30 litre capacity plastic bags held horizontally in light and temperature controlled tanks, has allowed mass production of the micro-alga for partial chemical characterisation of the toxic compound. Fractionation of the active extracts was guided by a haemolytic neutralisation assay (HNA) for palytoxin. Larval bioassays using sea urchins and Greenshell musselsTM (*Perna canaliculus*) indicated toxicity and extensive acute toxicological studies using small mammals confirmed toxicity by intraperitoneal injection. The assays, including comparative HNA results, indicate that the novel compound has an equivalent toxicity to palytoxin itself. *O. siamensis* cells were fed incrementally (46 x106 cells over three days) to shellfish and sea urchins in order to simulate bloom concentrations and to determine the risk to humans of seafood contamination. Results (HNA) were Greenshell mussels (whole shellfish), 14.5 µg/kg; scallops (minus hepatopancreas; *Pecten novaezealandiae*), 7.7 µg/kg; sea urchins, 1.2 µg/kg.

O.06-04
Effects of algal-produced neurotoxins on brain activity in atlantic salmon (*Salmo salar*)

Session: O.06 - Toxicology 1
Presentation time: 10:10 - 10:30

Marit Bakke, TE Horsberg

Norges Veterinærhøgskole, OSLO, Norway

In addition to being lethal at high concentrations, toxins released during harmful algae blooms may have implications for fish also in sublethal concentrations. The cellular mechanisms of the toxins used in this study are well described, however, little is known about the effects on the central nervous system in fish. This study aimed to look for changes in metabolic activity in different parts of the brain of Atlantic salmon after exposure to sublethal doses of three different neurotoxins. Seawater adapted postsmolts (93.4 g ± 1.9 g) were randomly selected to four groups for i.p. injection of saline (control) or one of the neurotoxins saxitoxin (10 µg STX/kg bw), brevetoxin (68 µg BTX/kg bw) or domoic acid (6 mg DA/kg bw). After 30 minutes, 14C-deoxyglucose (200 µCi/kg bw) was injected i.m., and the fish were sacrificed after an additional 30 minutes whereupon the head was instantly frozen in liquid propane. Sections of the brain (20 µm) were subjected to autoradiographical examination and the autoradiograms were analyzed digitally. Preliminary results suggest that all studied neurotoxins have an effect on the metabolic activity in various parts of the brain. Possible implications of the findings will be discussed.

O.07-01
Development of simple and rapid assays for diarrhetic shellfish toxins and yessotoxins based on enzyme inhibition and ELISA

Session: O.07 - Toxin analysis 1
Presentation time: 11:00 - 11:20

R Sekiguchi[1], M Suzuki[1], N Takahashi[1], M Yamamoto[1], M Watai[1], T Suzuki[2], T Yasumoto[1]

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[2]Tohoku National Fisheries Research Insti, SHIOGAMA, MIYAGI, Japan

In an ongoing project to develop screening methods suitable for on-site use, we evaluated the performance of our PP2A inhibition assay kits developed by using a catalytic subunit of PP2A from whelks. More than 500 samples of scallops and mussels were assayed and the resultant OA contents showed a good correlation (R2=0.9797) those obtained by LC-MS analysis. Next, we prepared a prototype microplate assay kit for YTXs using polyclonal anti-YTX antibodies. After optimizing the assay conditions, we assayed more than 500 samples and compared the results with thoset of LC-MS. A good linearity was observed between the ELISA and LC-MS results (R2=0.740). However, the values obtained by ELISA were 8 times higher than those calculated by LC-MS for YTX, indicating a higher cross-reactivity of 45-OH-YTX and other analogs against the antibody. Nevertheless, the good linearity observed between the two assays pointed to the usefulness of the ELISA assay as an on-site screening method for YTXs.

O.07-02
Comparison of the accumulation of lipophilic marine biotoxins in passive samplers, transplanted mussels and indigenous mussels on the Irish coast

Session: O.07 - Toxin analysis 1
Presentation time: 11:20 - 11:40

EE Fux, R Bire, P Hess

Marine Institute, ORANMORE, Ireland

The use of polymeric resin as passive sampling for an early warning system was recently published and referred to as Solid Phase Adsorption Toxin Tracking (SPATT). This technique involves the immersion of resin filled sachets in the seawater and was used during the summer 2005 on the west coast of Ireland in shellfish productions areas. Live mussels were placed next to the SPATT bags to compare toxins

accumulated in shellfish and onto the resin. Furthermore, all data obtained by LC-MS were compared to the toxin levels in indigenous mussels. The SPATT bags showed the ability to adsorb a wide range of lipophilic toxins (AZAs, OA, DTX2, PTX2, and YTX) even when no major toxic events occurred. The relocated mussels that were placed near the SPATT bags accumulated toxins (OA, DTX2, OA esters, DTX2 esters, AZA1 and AZA2) to a greater extent than the indigenous mussels. The SPATT bags allow the detection of toxins in the water but did not provide an early warning seven days prior the toxic event. The use of passive sampling had major advantages over current monitoring methods which use shellfish, as it enables the quantification of parent toxins rather than their metabolites and offered high sensitivity.

O.07-03
Genoa 2005 outbreak. Determination of a putative palytoxin in Mediterranean *Ostreopsis ovata* by a new LC/MS method

Session: O.07 - Toxin analysis 1
Presentation time: 11:40 - 12:00

P Ciminiello[1], C Dell'Aversano[2], E Fattorusso[2], M Forino[2], GS Magno[2], L Tartaglione[2], C Grillo[3], N Melchiorre[3]
[1]Università di Napoli 'Federico II', NAPOLI, Italy
[2]Università di Napoli 'Federico II', NAPOLI, Italy
[3]ARPAL, LA SPEZIA, Italy

During Summer 2005, symptoms of rhinorrhea, cough, fever, bronchoconstriction with mild dyspnea were observed in about 200 people exposed to marine aerosols on the beach of Genoa (Italy). The toxic outbreak was correlated with the co-occurrence of an unusual proliferation of the tropical microalga *Ostreopsis ovata*. Some *Ostreopsis* strains are proven to produce analogues of palytoxin. Thus, the need arose to develop an analytical method for detecting palytoxin in order to investigate whether the *O. ovata* blooming during the Genoa event was producing the toxin.
A new LC/MS method for palytoxin was set up on a turbo ionspray (TSI)- triple quadrupole MS instrument operating in selected ion monitoring (SIM) and multiple reaction monitoring (MRM) acquisition modes (positive ions). The minimum detection levels for matrix-free toxin on-column, which were estimated to be 17 pg (3.4 ng/mL) and 29 pg (5.8 ng/mL) in MRM and SIM mode, respectively, make the method suitable for monitoring program.
Application of the method to the analysis of a plankton sample collected along Genoa coasts during the toxic event, demonstrated for the first time the presence of a putative palytoxin in Italian waters and pointed to this toxin as the causative agent of the Genoa outbreak.

O.07-04
LC-MS for detection of paralytic shellfish poisoning (PSP) toxins in shellfish

Session: O.07 - Toxin analysis 1
Presentation time: 12:00 - 12:20

EA Turrell, L Stobo, J-P Lacaze
Fisheries Research Services, ABERDEEN, United Kingdom

The combination of hydrophilic interaction chromatography (HILIC) and tandem liquid chromatography-mass spectrometry (LC-MS/MS) provides a high degree of selectivity and sensitivity for the determination of paralytic shellfish poisoning (PSP) toxins (Dell'Aversano *et al*., 2005). At Fisheries Research Services, a HILIC LC-MS method for the detection of PSP toxins (STX, NEO, dcSTX, GTX1 to GTX4, dcGTX2 and dcGTX3) has been developed using a single quadrupole LC-MS for the detection of these toxins in UK shellfish. Calibration range and linearity were acceptable (0.5 to 5 µM, R2 > 0.99) and a detection limit of ~0.5 µM was achieved. Unfortunately, sensitivity was at least ten times less favourable when compared with validated high performance-liquid chromatography with fluorescence detection (HPLC-FLD), which can be prone to interference from endogenous fluorescent matrix compounds. In order to increase sensitivity of the LC-MS, we are currently developing methods for improved sample preparation, using solid phase extraction (SPE) and we will present our preliminary findings.

Dell'Aversano *et al*. / J. Chromatogr. A (2005) 190-201

O.07-05
Anatoxin contamination of freshwater resources in New Zealand

Session: O.07 - Toxin analysis 1
Presentation time: 12:20 - 12:40

PT Holland[1], AI Selwood[1], SA Wood[1], K Smith[1], P McNabb[1], P Rasmussen[2]

[1]Cawthron Institute, NELSON, New Zealand
[2]Australian Water Quality Centre, BOLIVAR, SA, Australia

Cyanobacterial blooms in New Zealand water resources have been surveyed and, in response to strict new standards for drinking water, more intensive monitoring for cyanotoxins has been initiated. A filamentous bloom-forming cyanobacterium from a lake has been shown to be a potent producer of anatoxin-a. Phylogenetic analysis of the 16S rRNA and rpoB gene sequences classified the organism as *Aphanizomenon issatschenkoi*. Benthic samples of *Oscillatoria* sp. and *Phormidium* sp. from several rivers were shown to produce anatoxin-a and homoanatoxin-a, and were conclusively associated with illness and death of dogs. These findings are the first confirmed reporting of anatoxin-producing species in the Southern Hemisphere and the first report of anatoxin-a production by *A. issatschenkoi*. An isolate of the latter was brought into culture and, during LC-MS assays for anatoxin-a production, an unstable biosynthetic precursor was found in fresh samples of the culture. Characterisation of the precursor is described and implications for accurate analysis of anatoxin-a in water samples are discussed. The New Zealand Drinking Water Standards (2005) include provisional maximum allowable values (PMAVs) for anatoxin-a, homoanatoxin-a, cylindrospermopsin and microcystins of 6, 2, 1 and 1 µg/L respectively. The validation of suitable test methods to enforce these low limits is presented.

O.07-06
The Biosense ASP ELISA - an early warning tool for the environmental monitoring of domoic acid in phytoplankton and seawater

Session: O.07 - Toxin analysis 1

Presentation time: 12:40 - 13:00

H Kleivdal[1], SI Kristiansen[1], C Campbell[2], K Davidson[2]

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[2]Scottish Association for Marine Science, OBAN, United Kingdom

The monitoring of potential harmful algae in coastal waters often relies on species determination and an estimation of their respective cell counts. There is no clear correlation between toxin levels and cell numbers for many toxin-producing species, and it is difficult to predict the effects of an upcoming bloom as some algae may be harmful at low abundance. A parallel routine determination of actual toxin levels is therefore important to complement the phytomonitoring as part of an effective early warning system. Until recently, the analysis of toxins in phytoplankton has been performed in centralized laboratories with costly and advanced instrumentation far from the sampling sites. As simple rapid assays for marine biotoxins become available, the analysis of phytoplankton and seawater samples can be performed close to the sampling site and provide a 'real time' monitoring of the toxin level in coastal waters. We have evaluated the Biosense ASP ELISA for direct analysis of domoic acid (DA) in seawater and several cultured and natural *Pseudo-nitzschia* sp. samples, using a simple sample preparation protocol. The ASP ELISA allows precise and accurate quantitation of DA at low concentrations in particulate and dissolved fractions, with an estimated limit of detection at 50 pg/mL.

O.08-01
Domoic acid production is not linked to silicate limitation in natural populations of *Pseudo-nitzschia*

Session: O.08 - Population dynamics 2
Presentation time: 11:00 - 11:20

WP Cochlan[1], ML Wells[2], VL Trainer[3], CG Trick[4], EJ Lessard[5], BM Hickey[5]

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[2]University of Maine, ORONO, United States of America
[3]Northwest Fisheries Science Center, NOAA, SEATTLE, United States of America
[4]Department of Biology, University of Wes, LONDON, Canada
[5]University of Washington, SEATTLE, United States of America

A central paradigm in the study of toxigenic diatoms is that silicate limitation induces intense cellular production of domoic acid. We tested this hypothesis during a 2004 ECOHAB-PNW cruise in the coastal waters off Washington State and British Columbia where toxigenic *Pseudo-nitzschia* typically occur. Our results show that the highest levels of cellular toxin (5-64 pg/cell) correlate poorly with ambient silicate concentrations and occur where dissolved silicate concentrations are 5-50 µM. None of the ~400 particulate analyses showed elevated cellular toxin concentrations at < 4 µM Si. These concentrations are well above that considered limiting for Si uptake and growth of most neritic diatoms. Cellular toxin levels also did not correlate with N or P concentrations, indicating that toxin production in I spp. is not governed by macronutrient availability. A similar result was found during the intense toxic *Pseudo-nitzschia* bloom in Monterey Bay, 1998. The most established correlate for elevated domoic acid levels in 2004 was the distribution of low dissolved iron concentrations; a finding consistent with laboratory culture experiments and our field incubation studies in this region. These findings provide perhaps the most detailed insight to date into the triggers for toxin production in natural populations of *Pseudo-nitzschia.*

O.08-02
The nature of the Juan de Fuca eddy: the rise and fall of domoic acid to the Washington State coast

Session: O.08 - Population dynamics 2
Presentation time: 11:20 - 11:40

VL Trainer[1], WP Cochlan[2], BM Hickey[3], EJ Lessard[3], A MacFadyen[3], CG Trick[4], ML Wells[5]

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[4]University of Western Ontario, LONDON, ONTARIO, Canada
[5]University of Maine, ORONO, ME, United States of America

The Juan de Fuca eddy is a retentive summer feature located off Washington State, U.S.A. whose location and size is dependent on wind as well as freshwater outflow from the adjacent strait. Although domoic acid-producing *Pseudo-nitzschia* species have been recorded in this region since 1996, their abundance and toxicity vary from year to year in a manner that is at present unpredictable. Over the last 3 years, ECOHAB Pacific Northwest researchers have performed 5 synoptic cruises to characterize the strength and variation in the biological, physical and chemical factors pertinent to HAB success. The interdisciplinary data indicate that the eddy can be contrasted to the coastal upwelling zone by the following: (1) higher specific cellular toxicities, especially in *Pseudo-nitzschia* found around its edges, (2) a greater degree of iron limitation (3) a more persistent macronutrient supply. The shifting physiological health of *Pseudo-nitzschia* cells in transit from the eddy also points to its uniqueness. In spite of a well developed eddy in September 2004 featuring high levels of both particulate (up to 95 nM) and dissolved domoic acid (up to 20 nM), this HAB never reached the coast, illustrating that toxic events require both toxin production and onshore transport.

O.08-03
The role of grazer induced toxin production in harmful algal bloom formation

Session: O.08 - Population dynamics 2
Presentation time: 11:40 - 12:00

E Selander, A Arnqvist, J Bergkvist, H Pavia

Göteborg University, STRÖMSTAD, Sweden

Waterborne cues from several species of copepod grazers induce increased paralytic shellfish toxin (PST) production in the dinoflagellate *Alexandrium minutum*. The induction is density dependent and more PST is formed when more waterborne cues from grazers is present. Here, water borne cues from the copepod grazer Acartia tonsa was used to manipulate cell specific PST content in cultures of *A. minutum*. The resulting *A. minutum* cultures ranged in toxicity from 8 fmol PST cell^{-1} in control cultures to 48 fmol PST cell^{-1} in cultures that received most waterborne cues from grazers. The *A. minutum* cultures of different

toxicity were subsequently used in feeding preference experiments with copepod grazers and different non-toxic alternative phytoplankton prey. The results showed that copepods discriminated against more toxic *A. minutum* and significantly less A. minutum was ingested in treatments were induced (more toxic) *A. minutum* were present. Discriminate feeding of copepods resulted in a shift towards A. minutum dominated species composition in experiments with induced, more toxic, *A. minutum*. It is suggested that induced resistance to grazers may contribute to the formation of harmful algal blooms.

O.08-04
The impacts of viral infection on *Heterosigma akashiwo* blooms

Session: O.08 - Population dynamics 2
Presentation time: 12:00 - 12:20

JE Lawrence
University of New Brunswick, FREDERICTON, Canada

A number of viruses infect the harmful bloom-forming raphidophyte *Heterosigma akashiwo* such as HaNIV, HaRNAV and a novel system in which two distinct viruses co-infect a host cell. These lytic viruses are distinct in their biochemical composition; they contain different nucleic acids and proteins and are therefore genetically diverse. Lytic infections in general are marked by a number of events: replication of progeny, lysis of the host and the concomitant release of progeny and organic material. This cycle inherently means viruses impact host abundance and nutrient cycling. However, a number of factors are specific to viruses and therefore result in diverse impacts of viral infection in phytoplankton population dynamics in situ. For example the lytic cycle length, burst size and bloom density interact to influence the rate of propagation of infection, and therefore the development or demise of *H. akashiwo* blooms. In addition, the host range of each virus clone and permissiveness of each host strain shape the genetic composition of *H. akashiwo* populations. These factors will be examined and used to demonstrate the role of virus in phytoplankton blooms, and the importance of isolating and characterizing novel viruses to understand the role of viruses in phytoplankton mortality.

O.08-05
Trigger factors of *Alexandrium catenella* blooms in Hong Kong

Session: O.08 - Population dynamics 2
Presentation time: 12:20 - 12:40

KC Ho
Open University of Hong Kong, HONG KONG, Hongkong

The environmental data collected in Hong Kong waters during the past 20 years were reviewed and statistically analysed. Results showed that the ranges of water temperature, solar intensity, salinity and wind and current directions during the periods of *Alexandrium catenella* bloom were significantly ($p<0.05$) different from the normal ranges of these parameters during the winter of Hong Kong as well as the ranges of these parameters during the red tides of other dinoflagellates. While it is not possible to correlate the concentrations of TIN in seawater with the blooms of A. catenella, results obtained from bottle-bioassay in environmental chambers revealed that the yield of A. catenella was maximized when ambient TIN ranged from 0.05-0.1mg-N/L and N:P atomic ratio ranged from 18-22. Analysis of meteorological data shows that blooms of *A. catenella* happened at early February to March when the coastal water of Hong Kong is prominent by the intruded oceanic water from Taiwan Strait. It is suggested that the vegetative cells of *A. catenella* were seeded from the Kuro Shio Current and blooms was triggered by the relatively low temperature (18-22 °C) and relative high N:P atomic ratio in coastal embayment.

O.08-06

A massiv bloom of *Alexandrium fundyense* in the Gulf of Maine: mechanisms and future implications

Session: O.08 - Population dynamics 2
Presentation time: 12:40 - 13:00

DM Anderson[1], BA Keafer[1], K Norton[1], R He[1], DJ McGillicuddy, Jr[1], CH Pilskaln[2], D Couture[3]

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A massive bloom of *Alexandrium fundyense* affected southern New England in 2005, closing nearshore shellfish beds from Maine to Massachusetts and 40,000 km^2 of offshore federal waters. This was the largest regional bloom in over 30 years. Observations suggest three factors in bloom development: 1) historically high cyst abundance; 2) strong storm activity with northeast winds; and 3) heavy rainfall and snowmelt inputs. A physical/biological model was used to hindcast the bloom and investigate the relative importance of these factors. Simulations indicate that high cyst abundance was a critical factor in the bloom's size, perhaps the most important of the three. Wind forcing was also important, however, as episodic bursts of northeast winds accelerated along-shore flow and caused onshore advection of offshore populations. While affecting cell abundance in southern waters, buoyant plumes formed from elevated river runoff were confined to the coast and had limited impact on the broad, gulf-wide bloom distribution. Based on historical toxicity patterns in shellfish and high numbers of cysts observed in the region in 2004 and 2005 (pre- and post bloom), we hypothesize a 'new regime' with more frequent and intense toxicity in western Maine and southern New England over the next decade or more.

PL.04

Allelopathic effects of bioactive compounds produced by harmful algae

Session: PL..04 - Plenary IV - Allelopathy
Presentation time: 14:15 - 14:50

Urbann Tillmann, U John, B Krock, A Cembella

Alfred Wegener Institute, BREMERHAVEN, Germany

Allelopathy may be defined as 'any process involving secondary metabolites produced by plants, microorganisms, virus and fungi that influence the growth and development of natural systems' (International Allelopathic Society). Such chemical interactions have been widely studied in terrestrial ecology but are less well known in

marine ecosystems, especially among protists. In the marine plankton, temporal and spatial changes of biomass and species composition have traditionally been thought to be mainly regulated by resource availability and abiotic factors. However, there is increasing evidence that inter-specific interactions in the plankton play a major role in succession, food web structure and bloom development. Many HAB species are regarded as rather poor exploitation competitors in terms of growth rate and/or resource uptake capabilities. There is some evidence for the hypothesis that a number of HAB species may gain dominance by the production of bioactive compounds, secondary metabolites that affect growth or elicit other physiological responses in other organisms. Such allelochemicals may be targeted to exclude competitors from exploiting limited resources (interference competition), as well as to avoid/reduce predation. HABspecies with allelopathic potency include various cyanobacteria, haptophytes and dinoflagellates. Although the causative substances are poorly described, such allelochemicals seem to be distinct from the commonly known phycotoxins.

O.09-01

Marine phytoplanton allelochemicals affect growth and composition of bacterioplankton

Session: O.09 - Allelopathy
Presentation time: 14:55 - 15:15

C Legrand[1], T Bouvier[2]
[1]University of Kalmar, KALMAR, Sweden
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Chemical interactions among marine microbes are an adaptation to avoid predation and to eliminate competitors. Recently, the importance of allelopathic interactions among phytoplankton and heterotrophic protists has been demonstrated. Potentially, phytoplankton allelochemicals could also affect other microbes. The traditional view is that bacterioplankton benefit from DOM produced by phytoplankton. However, during high density algal blooms, bacterial biomass is often lower than expected in relation to algal DOM. We tested the hypotheses that phytoplankton allelochemicals would affect bacterioplankton composition and growth. The allelopathic effect of the prymnesiophyte *Prymnesium parvum* was tested on a wide range of bacterial isolates belonging to 5 different groups and on natural populations. Results showed that algal allelochemicals selectively inhibited different bacterial targets with different efficiency, ranging from 5-10% for Actinobacteria to >60% Proteobacteria. Bacteroidetes were not affected by allelochemicals. The allelopathic effect on natural bacterial communities was proportional to the concentration of allelochemicals, indicating that the inhibition of bacterioplankton may increase during bloom formation. The composition of natural bacterioplankton was significantly affected by allelochemicals. We conclude that algal allelochemicals must be considered, along with resources, bacterivory and viral lysis, as a fundamental control factor of marine bacterial growth, composition and potentially also activity.

O.09-02
Allelopathic interactions modulate brevetoxin production in the red tide dinoflagellate *Karenia brevis*

Session: O.09 - Allelopathy
Presentation time: 15:15 - 15:35

J Kubanek[1], EK Prince[1], T Myers[1], J Naar[2]

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The production and release of compounds that inhibit competitors, a process known as allelopathy, is hypothesized to be important among phytoplankton, especially those that occur in dense blooms. We previously found that the red tide dinoflagellate *Karenia brevis* is allelopathic towards several Gulf of Mexico competitors, and that brevetoxins are responsible for suppressing one competitor, *Skeletonema costatum*. Recent laboratory and field experiments have led to the finding that *S. costatum* retaliates by inhibiting brevetoxin production in *K. brevis*. Natural bloom samples of *K. brevis* lose their allelopathic effect when exposed to *S. costatum*. Overall, chemically-mediated competitive interactions in the marine plankton appear to be complex, multi-directional, and part of an ongoing co-evolutionary battle over limiting resources.

O.09-03
Allelopathy in *Karenia mikimotoi*: a case study

Session: O.09 - Allelopathy
Presentation time: 15:35 - 15:55

Patrick Gentien

IFREMER, PLOUZANÉ, France

*Karenia mikimoto*i is a widely distributed potent fish killer. This contribution summarizes the works conducted on its allelopathic properties. This property has been demonstrated in laboratory cultures and confirmed in situ. The minimum level for repression in diatom growth was estimated ca. 10^4 cells · l^{-1}, indicating that allelopathy can favour the bloom development. One of the allelopathic agents (a labile fatty acid : 18:5n3) was identified in the cells and in the cultures media. To understand the mode of action of the toxic agent, it was synthetized from 22:6n3. This fatty acid inhibits in a non-specific way membrane ATPases, rendering biological targets unable to regulate their ion exchanges. The short half-life time (30 min) of this toxin explains that it doesn't saturate the medium and that cells transport their allelopathic. Scaling arguments lead to an estimation of a action sphere around each cell is ca. 175 µm. The consequence is that allelopathy is acting only by proximity. It was recently demonstrated that a trade-off has been established between the advantage gained by allelopathy and the sensitivity of *K. mikimotoi* to its own allelopathic agent. Some general conclusions are proposed on the basis of this case study.

O.09-04
Spirolide variability and biological activity of natural products from the marine dinoflagellate *Alexandrium ostenfeldii*

Session: O.09 - Allelopathy
Presentation time: 15:55 - 16:15

AD Cembella[1], C Kantu[2], B Krock[1], N Jaeckisch[1], E Cañete[3], A Caillaud[3], J Diogène[3], U Tilmann[1], U John[1]
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The marine dinoflagellate *Alexandrium ostenfeldii* produces a wide diversity of macrocyclic imine toxins known as spirolides. Biosynthetic studies with stable isotope labeling and NMR spectroscopy support a common polyketide origin for spirolides. We have also identified several putative polyketide synthases (PKS) in limited genomic studies of *A. ostenfeldii*. Exposure to *A. ostenfeldii* cells elicits an allelochemical response in certain protist species. To determine pharmacological activity of spirolides and other bioactive substances produced by A. ostenfeldii, neuroblastoma (N2a) cell lines were exposed to crude cell extracts and pure des methyl C spirolide. Cytotoxicity tests showed that crude extracts caused large blebbings within hours in a dose-dependant manner. Cells exposed to des methyl C spirolide induced only small blebbings, with no further changes observed over longer exposure times (24 h).
Transcriptional analysis with a pathway targeted DNA microarray yielded evidence of many more 'down regulated' than 'up regulated' genes upon exposure to spirolides and crude cell extracts, particularly genes involved in necrosis/apoptosis/stress (e.g. 'heat shock proteins') and growth/senescence. Nevertheless, the differences observed in expression patterns between des methyl C spirolide and spirolide-containing *A. ostenfeldii* extracts suggest that the toxic effects are not due only to spirolides but also to other bioactive substances.

O.10-01
Successful production of antibodies against azaspiracids

Session: O.10 - Toxin analysis 2
Presentation time: 14:55 - 15:15

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Azaspiracids have recurred regularly in Northern European shellfish since their discovery in November 1995 in Irish mussels. Their presence in shellfish poses a risk of acute nausea, vomiting and severe diarrhoea in human consumers and chronic effects are suspected. There is an urgent need for sensitive, simple, rapid, affordable methods with a high sample throughput. Here we report the first production of antibodies against azaspiracids.
A synthetic hapten representing the common C-28–C-40 domain of all the known azaspiracids was synthesised in Minnesota, conjugated to a carrier protein, and used to immunise sheep for antibody production in both Norway and New Zealand. Preliminary results indicate that the resulting antibodies recognise a range of azaspiracids. The antibodies have been utilised to develop an ELISA for azaspiracids and immunoaffinity columns. Further optimisation of the application of the antibodies in immunoassays is under way.

Progress to date and potential applications will be summarised.

O.10-02

Laboratory evaluation and method development of solid phase adsorbents for hydrophilic phycotoxins in marine and freshwater applications

Session: O.10 - Toxin analysis 2
Presentation time: 15:15 - 15:35

A Robertson, KL Reeves, J Capling, C Garnett, MA Quilliam

National Research Council of Canada, HALIFAX, Canada

Solid phase adsorption toxin tracking (SPATT) is a relatively new technology that was developed to facilitate routine monitoring of lipophilic toxins. We have now sought to evaluate adsorbents that could be applied to SPATT for hydrophilic phycotoxins including paralytic shellfish toxins (PSTs) and domoic acid (DA). During laboratory scale experiments, we investigated the removal of a variety of PSTs from water using three different activated carbons (ActCs). Cell free extracts of *Alexandrium tamarense* were added to both distilled water and filtered seawater. All ActC subtypes tested effectively removed PSTs from the water including STX, NeoSTX, dcSTX, GTX 2, GTX 3, dcGTX 2, dcGTX 3 and C-toxins. Likewise, water spiked with DA-containing shellfish extracts resulted in complete adsorption to the ActC. To optimize toxin recoveries, a variety of extraction procedures were examined and one ActC subtype proved promising for subsequent experiments. This ActC had the advantage of combining toxin capture, concentration and clean-up in one step with consistent recovery of individual PSTs. In contrast, sub-optimal recoveries were observed for DA therefore a variety of alternative adsorbents were assessed. We aim to validate the use of these adsorbents for sample clean-up strategies, potable water applications and to complement HAB monitoring programs.

O.10-03

Karenia concordia* (Dinophyceae) as a brevetoxin-producer and comparison with two closely related species *K. brevisulcata* and *K. mikimotoi

Session: O.10 - Toxin analysis 2
Presentation time: 15:35 - 15:55

FH Chang[1], AJ Bourdelais[2], DG Baden[2], M Gall[1], D Hulston[1], V Webb[1]

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In New Zealand a non-thecate dinoflagellate, *K. concordia*, reported as a causative organism of the 2002 toxic events on the north-east coast, has for the first time been unequivocally confirmed as a brevetoxin-producer. Subsequent analysis using high resolution LC-MS revealed the presence of five brevetoxin derivatives, with Pb-Tx-2 as the dominant toxin. Using HPLC technique, pigments of *K. concordia* were compared with two closely related species, *K. brevisulcata* and *K. mikimotoi*. The pigment profile of K. concordia differed from the other two species, but was most like that of *K. brevisulcata*. Toxins of all three species elicited distinct toxicological responses from neuroblastoma single cell lines and organisms from six different classes of microalgae to brine shrimps. Although all three dinoflagellate species exhibited haemolytic activities, toxins produced by *K. concordia* and *K. brevisulcata* acted much faster than that of K. mikimotoi. Unlike *K. mikimotoi*, both *K. concordia* and *K. brevisulcata* are lethal nearly to all organsims tested. The potential for using the bioassays developed as diagnostic tests for the different haemolytic/neurotoxic toxins are discussed.

O.10-04
Impact of environmental factors on growth, toxicity and toxin production of harmful algae *Chattonella marina* (Kagoshima strain)

Session: O.10 - Toxin analysis 2
Presentation time: 15:55 - 16:15

Shahroz M Haque

Bagladesh Agricultural University, MYMENSINGH, Bangladesh

Growth, toxin profile and intensity of toxicity of the red tide-forming phytoflagellate *Chattonella marina* (Kagoshima Strain) were investigated at different temperatures and light intensities. The optimal growth range was at 25-30 °C and a light intensity of 100-150 µmol $m^{-2} s^{-1}$. Ichthyotoxicities at different temperatures and light intensities were found to vary immensely, the highest toxicity was found at 20 °C and 150 µmol $m^{-2} s^{-1}$. *Chattonella marina* contained the toxin components CmTx-I, CmTx-II, CmTx-III and CmTx-IV, corresponding to PbTx-2, PbTx-9, PbTx-3 and oxidized PbTx-2. Toxin yields varied markedly with temperature and light intensity. The toxic components CmTx-I, CmTx-II and CmTx-III peaked at 20 °C with yields of 0.35, 0.30 and 2.5 pg $cell^{-1}$, but the highest yield (0.7 pg $cell^{-1}$) of CmTx-IV was at 30 °C. The yields of all CmTx components decreased sharply at temperatures exceeding 20 °C. The highest amount of the toxin CmTx-IV (0.70 pg $cell^{-1}$) was also at 150 µmol $m^{-2} s^{-1}$. A negative correlation between growth rate and toxin production was found in the strain.

O.11-01
The genetic basis for the biosynthesis of PSTs in cyanobacteria and algae

Session: O.11 - Genetics
Presentation time: 16:40 - 17:00

R Kellmann[1], YJ Jeon[2], TK Mihali[2], R Cavaliere[2], BA Neilan[2]

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[2]University of New South Wales, SYDNEY, Australia

Research efforts are growing worldwide in an attempt to understand the ecophysiological mechanisms involved in the formation, control and spread of toxic blooms, however little is known about the biosynthesis of algal toxins, the identity of genes and enzymes involved, and how they are regulated. Saxitoxin and its analogues, which are responsible for paralytic shellfish poisoning, are one of the most enigmatic groups of algal toxins. Although they are synthesised by a complex and unique pathway, organisms from two kingdoms, which are the dinoflagellates and cyanobacteria, are capable of producing the same toxins. In spite of efforts over the last 30 years, the identity of genes involved in this pathway have remained a mystery. Here we present the genes that are responsible for the biosynthesis of saxitoxin analogues in cyanobacteria from the genera

Anabaena, *Cylindrospermopsis*, *Aphanizomenon*, and *Lyngbya*. The evolution and possible horizontal transfer of these genes between cyanobacteria and dinoflagellates, and how the production of these toxins is regulated on the genetic level have also been investigated. Based on the structural genomic information and the catalytic activities of heterologously expressed saxitoxin biosynthesis genes, the previously proposed biosynthetic pathway for saxitoxin has been revised.

O.11-02
Microsatellite markers reveal population genetic structure in the noxious red tide-causing algae *Heterosigma akashiwo* (Raphidophyceae) in Japanese coastal waters.

Session: O.11 - Genetics
Presentation time: 17:00 - 17:20

Satoshi Nagai[1], S Yamaguchi[1], CL Lian[2], Y Matsuyama[1], S Itakura[1]

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We isolated 13 polymorphic microsatellites from the noxious red tide-causing algae, Heterosigma akashiwo. These loci provide a class of highly variable genetic markers as the number of alleles ranged from 3 to 12 and the observed and expected heterozygosity ranged from 0.286 to 0.926 and from 0.314 to 0.888, respectively. Each locus showed either one or two bands for each individual, indicating homozygous or heterozygote state in a diploid. Here we present the genetic analysis of *H. akashiwo* populations from several sites along the Japanese coastal waters. We finished the microsatellite analysis at 3 areas of Japan thus far and the analysis showed that highly significant differentiation was detected in all 3 pairwise comparisons after Bonferroni correction in the Fisher's test and that genetic distance (Nei 1972) was ranged from 0.27 to 0.61, showing remarkably higher than those seen in *Alexandrium tamarense* populations (0.07-0.29). These results clearly indicate that genetic isolation and restricted gene flow via natural dispersal through tidal currents has occurred among the populations. The microsatellite analysis of another local populations is now undertaken and we would like to present the more detail information on genetic structure in *H. akashiwo* populations in Japanese costal waters at the conference.

O.11-03
Genetic differentiation and phenotypic characteristics of geographically separated populations of the *Alexandrium tamarense* North American ribotype

Session: O.11 - Genetics
Presentation time: 17:20 - 17:40

T Alpermann[1], U John[1], U Tillmann[1], KM Evans[2], S Nagai[3], DM Anderson[4], AD Cembella[1]

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[2]Royal Botanic Garden, EDINBURGH, United Kingdom
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[4]Woods Hole Oceanographic Institution, MASSACHUSETTS, United States of America

The '*Alexandrium tamarense* species complex' contains prominent paralytic shellfish poisoning (PSP) toxin producers that can be further discriminated either on the basis of morphological characteristics or by DNA sequence divergence. The North American clade, as defined by its LSU ribosomal DNA sequence, is the most widely distributed representative of the PSP toxin-producing *A. tamarense* clades. Populations of this clade cause recurrent blooms in many regions of the world. Natural populations from North America, Northern Europe and Japan exhibit notable genetic differentiation that can be detected by molecular markers with different resolution properties. Whereas molecular sequence analysis of ribosomal DNA yields only a coarse resolution pattern of regional subclades, mitochondrial DNA sequences and microsatellites, as well as Amplified Fragment Length Polymorphism (AFLP) analysis, allow the estimation of genetic differentiation between contiguous populations. No congruence of any of the genetic markers were found between the expression of PSP toxin phenotypes or allelochemical properties that can affect grazers or competing algal species, but inter-population differences in PSP toxin profile were apparent on a broad geographical scale. The variable expression of the allelopathic phenotype within a population from Northern Europe was used to experimentally test the protective benefit of allelochemical properties on bloom formation.

O.11-04
Genetic differences between *Karlodinium veneficum* strains: using DNA variation to understand strain variation at the bloom, regional and worldwide level

Session: O.11 - Genetics
Presentation time: 17:40 - 18:00

TR Bachvaroff

UMBI, BALTIMORE, United States of America

Karlodinium veneficum is a toxic bloom forming dinoflagellate found in estuarine systems throughout the world. Strains from the U. S. Atlantic coast (24 strains), the North Sea (3) and the Pacific (3) as well as a single Mediterranean strain were used to understand the genetic basis of the wide range of karlotoxin types and cellular quotas observed among strains of *K. veneficum*. The phenotype is expressed as differences in toxin (pg) per cell, toxin type, and pigment to chlorophyll ratios. Differences in genotype were determined using genetic loci isolated from a library of enriched repeat sequences. The ITS sequence and pigment ratios can distinguish between U.S. Atlantic coast strains and European or Pacific isolates. Simple sequence repeats, or microsatellite loci can distinguish among the U.S. strains. Further, one marker, KvF4 yields a 485 bp fragment for U.S. Chesapeake Bay strains producing karlotoxin 1 while the same locus yields a 283 bp fragment for all other US strains producing karlotoxin 2. Generally, these genetic markers can be divided into those useful for distinguishing between different isolates from the same bloom, those that distinguish strains from the same region, and those that distinguish between strains from different parts of the world.

O.12-01
In vivo exposure to microcystins induced DNA damage in haemocytes of the zebra mussel as measured with the Comet assay

Session: O.12 - Toxicology 2
Presentation time: 16:40 - 17:00

Guillaume Juhel[1], J O'Halloran[1], SC Culloty[1], RM O'Riordan[1], J Davenport[1], NM O'Brien[2], KF James[3], A Furey[3], O Allis[3]

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[2]2Department of Food Science, Food Techno,, Ireland
[3]Cork Institute of Technology, CORK, Ireland

The Comet assay was used to investigate the potential of microcystins to induce DNA damage in the haemocytes of the freshwater zebra mussel, *Dreissena polymorpha*. Laboratory in vivo exposure experiments were conducted over a 21-day period with three strains of the cyanobacterium *Microcystis aeruginosa*, with different toxicities and toxic profiles and one non-toxic strain. Mussels were sampled at 0, 7, 14 and 21 days. A positive control was performed with CdCl2-spiked water.
Cell viabilities were high throughout the study, demonstrating that the microcystin doses were not cytotoxic. A clear dose-response in the DNA damage was observed following exposure to CdCl2, showing the sensitivity of the mussels' haemocytes. DNA damage, measured as percentage tail DNA was observed with the three toxic *Microcystis* strains but not with the non-toxic strain. Toxic analysis of the cyanobacterial cultures revealed the presence of two MC variants, MC-LF and MC-LR. The DNA damage observed appeared to be strain-specific and similar to a dose-response, showing that MC-LF may have a higher genotoxicity than MC-LR. MC-LF also seemed to induce more persistent DNA damage than MC-LR. This study is the first to demonstrate that in vivo exposure to microcystins induces DNA damage in the haemocytes of zebra mussels.

O.12-02
Toxic effects of nodularin to the brown alga *Fucus vesiculosus* in relation to oxidative stress response

Session: O.12 - Toxicology 2
Presentation time: 17:00 - 17:20

Stephan Pflugmacher[1], Harri T Kankaanpää[2], Miika Olin[2]

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[2]Finish Institute of Marine Research, HELSINKI, Finland

The cyclic pentapeptide nodularin (NOD) is produced by the cyanobacterium *Nodularia spumigena* which regularly form heavy blooms in the Baltic Sea during summer season. The most common NOD congener is NOD-R, i.e. cyclo(-D-erythro-?-methylAsp(iso)-L-Arg-Adda-D-Glu(iso)-2-(methylamino)-2-(Z)-dehydrobutyric acid), where Adda stands for 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid. Recent studies have indicated that cyanobacterial hepatotoxins accumulate to blue mussels and some Baltic fish species, e.g. flounder (*Platichthys flesus*), Atlantic cod (*Gadus morhua*) and blue mussel (*Mytilus edulis*) and threespine stickleback (*Gasterosteus aculeatus*). A compartment of the marine costal ecosystem which has been paid less attention is marine macroalgae. The uptake of toxin in *F. vesiculosus*, toxic response (lipid peroxidation) as well as the activation of the antioxidative stress response (superoxide dismutase, catalase, and glutathione S-transferase) is shown.

O.12-03
Cytotoxic and genotoxic effects of microcystins in mammalian cell lines.

Session: O.12 - Toxicology 2
Presentation time: 17:20 - 17:40

E Dias[1], P Pereira[1], C Batoréu[2], P Jordan[1], MJ Silva[1]
[1]National Health Institute, LISBON, Portugal
[2]Faculty of Pharmacy of Lisbon University, LISBON, Portugal

Microcystin-LR (MCLR) has been recognized as a tumor promoter. However, knowledge of its carcinogenicity mechanisms remains largely unknown. In this work we evaluated the genotoxic potential of microcystins in mammalian cell lines. Microcystins were extracted from *M. aeruginosa* and purified by preparative liquid chromatography. Cytotoxicity tests (MTT reduction, LDH release) were used to determine the sensitivity of several cell lines (Vero, HepG2 and AML12) to MCLR (1.4-175 μg ml^{-1}). Genotoxicity of MCLR (2-60 μg ml-1) in Vero cells were analyzed by the micronucleus assay. MTT and LDH assays showed that MCLR had dose- and time-dependent cytotoxic effects on all three cell lines. However, Vero cells were the most sensitive, showing a 50% decrease in the viability after exposure to 22 μg ml^{-1}of MCLR for 24h. Above 48h, cell survival decreased more than 80%. Similar effects were obtained with the other cells after longer exposure (48-96h) to higher toxin concentrations (44-175μg ml^{-1}). Preliminary results of micronuclei analysis in MCLR-treated Vero cells (= 20 μg ml^{-1}) revealed an aneugenic/clastogenic activity. In summary, we identified a permanent cell line as a useful model system for microcystins toxicity/genotoxicity assessment and we showed that MCLR presents genotoxic properties. This supports previous suggestions that microcystins can act as cancer initiators.

O.12-04
Ecological implications of cylindrospermopsin in freshwaters

Session: O.12 - Toxicology 2
Presentation time: 17:40 - 18:00

M Seifert
University of Queensland, INALA, Australia

The potent cyanobacterial toxin, cylindrospermopsin, is being increasingly found in freshwaters around the world. In addition, the production of the toxin, and its analogues deoxy-cylindrospermopsin and 7-epi-cylindrospermopsin, is being reported from an increasing number of cyanobacteria. In Queensland (Australia), *Cylindrospermopsis raciborskii* is the dominant cylindrospermopsin producing cyanobacterium and has been implicated as the causative agent in an instance of mass human intoxication, numerous livestock mortalities and is considered a major threat to drinking water supplies. This has lead to substantial research into the cylindrospermopsin production, the removal of the toxin from drinking waters and its toxicity to mammals. In contrast, the potential ecological implications of C. raciborskii and cylindrospermopsin have been largely ignored. The Queensland Department of Natural Resources, Mines and Water recognised this

knowledge gap and allocated funds for a PhD project to investigate some of the ecological effects of cylindrospermopsin. This presentation will summarise these findings in the context of an ecological risk assessment.

PL.05
Anthropogenic influence and climatic change: effects on harmful algae

Session: PL..05 - Plenary V
Presentation time: 08:30 - 09:05

Barrie Dale

Dept of Geosciences, University of Oslo, OSLO, Norway

Understanding the possible effects of anthropogenic influence and climate change is a critical requirement in developing the risk assessments needed for the effective management of harmful algal blooms (HABs). However, the scientific issues involved are complex, and the relevant evidence is sparse, resulting in more uncertainty than understanding. There are uncertainties concerning HABs (e.g. are HABs increasing, and what are the underlying factors that allow some species to bloom preferentially?). There are also great uncertainties regarding the extent of human impact on aquatic environments (e.g. it is often difficult to differentiate the effects of eutrophication, pollution, over-fishing, etc.). The one thing we are certain of regarding climate is that it is changing – and it always has. There is a general consensus that global warming is occurring, but great uncertainty in predictions of future temperature increases. These range from 1-2^{o} (similar to the Medieval Warm Period, 550-1300AD), to up to several times more, with potentially devastating effects from ice-cap melting and global flooding. Uncertainties affecting assessment of environmental change and its effect on HABs are mostly caused by the lack of adequate time series data. The sedimentary record of microfossils offers one of the few plausible methods for redressing this shortfall.

O.13-01
Different seeding strategies within harmful algal bloom (HAB) causing dinoflagellates and diatoms

Session: O.13 - Life cycles
Presentation time: 09:10 - 09:30

Shigeru Itakura, S Nagai, M Yamaguchi
FEIS, HIROSHIMA, Japan

Dinoflagellates and diatoms are common phytoplankters in coastal marine environments and many HAB causing species are included in these two groups. They are known to produce benthic resting stages (resting stage cells) such as resting cysts, resting spores and resting cells in their life cycle. Benthic resting stages are considered to play an important role in their local reoccurrence. Under fluctuating coastal environmental conditions, the timing of germination is a key factor in their seeding strategy. Newly formed resting stages are in a state of endogenous dormancy. So, they need to be matured (get rid of the dormant state) prior to their germination. However, even in matured resting stage cells, germination is inhibited under unsuitable environmental conditions. Water temperature, light intensity and dissolved oxygen seem to have a decisive role in the germination of matured resting

stage cells. In general, diatom resting stages are able to germinate under a wider temperature range than dinoflagellates cysts, whereas dinoflagellate cysts are able to germinate under lower light intensity levels than diatom resting stages. In this talk, the different physiological responses to environmental factors within dinoflagellate/diatom resting stages will be examined and the significance of different seeding strategies will be discussed.

O.13-02
The life cycle of *Pyrodinium bahamense* var. *compressum* (Dinophyceae) in culture

Session: O.13 - Life cycles
Presentation time: 09:30 - 09:50

Setsuko Sakamoto[1], M Yamaguchi[1], EF Furio[2]

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[2]NFRDI, QUEZON, Philippines

Pyrodinium bahamense var. compressum has resting cyst stage in its life cycle. However, several processes in the life cycle are obscure, because most of previous studies based on observation of the natural populations. In present study, we investigated cyst formation and germination processes of *P. bahamense* in culture and revealed that the cysts were formed by sexual reproduction and the sexuality was homothallic. Resting cysts were spherical to ovoid and covered with many long processes. The cysts were formed in not only the crossing cultures but in the clonal cultures after over 35 days of incubation under 30 °C. Both crossing and clonal cysts were not able to distinguish by the cell size and the external morphology each other. Relative DNA contents of the resting cysts were 2C DNA while the vegetative cells were 1C DNA. Both crossing and clonal cysts germinated at 30 °C under light and dark cycle after 2.5-5 months. Therefore dormancy period is necessary at least 2.5 months for germination of *P. bahamense* cyst. On the basis of the results, schematic representation of the life cycle of *P. bahamense* var. *compressum* will be presented.

O.13-03
Life cycle traits and population dynamics of *Pseudo-nitzschia multistriata* in the Gulf of Naples (Mediterranean Sea)

Session: O.13 - Life cycles
Presentation time: 09:50 - 10:10

D D'Alelio, A Amato, A Lüdeking, M Ribera d'Alcalà, D Sarno, A Zingone, M Montresor

Stazione Zoologica 'Anton Dohrn', NAPOLI, Italy

The potentially toxic diatom *Pseudo-nitzschia multistriata* has been recorded in the Gulf of Naples since 1996. The species occurs from late summer to early spring, with two periods of major abundance (up to 200 cells ml^{-1}) in late summer-autumn and in early winter. Within the EU Project SEED, the potential role of life cycle traits in the population dynamics of the species is investigated. In this paper, data are presented on genetic diversity, size reduction and mode of sexual reproduction. Within the species two ITS genotypes are identified, which co-occur in the same period of the year. Sexual reproduction requires the pairing of distinct mating types. The auxospore allows the re-establishment of the maximum size (ca. 80 µm), while progressive cell-size reduction down to 30 µm

occurs during vegetative growth. Size distribution observed in natural populations throughout a 10-year time series shows cell length ranging between 30 and 80 µm. Three sub-populations with size modes of ca 60, 45, and 35 µm alternate over the years. Based on size reduction rates and growth characteristics, a model is proposed aimed at reproducing life cycle features and the timing of sexual events of *P. multistriata* at sea.

O.13-04
Asexual and sexual reproduction in *Protoperidinium steidingerae* (Dinophyceae)

Session: O.13 - Life cycles
Presentation time: 10:10 - 10:30

Kristin E Gribble[1], D Wayne Coats[2], Donald M Anderson[1]

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Protoperidinium is a cosmopolitan genus of heterotrophic dinoflagellates with more than 200 species. One species, *P. crassipes*, has been implicated as the source of azaspiracid shellfish toxin. In this study, division, sexuality, mandatory dormancy and germination rates of hypnozygotes, and identity life-history stages were revealed for the first time for any *Protoperidinium* spp. Asexual division occurred by eleutheroschisis within a temporary, immotile cyst, yielding two daughter cells. Daughter cells were initially round and half to two-thirds the size of parent cells, then rapidly increased in size, forming horns before separating. Sexual reproduction was constitutive in both non-clonal and clonal cultures, indicating that the species may be homothallic. Gametes were isogamous, approximately half the size and lacking the pink pigmentation of the vegetative cells, and were never observed to feed. Gamete fusion resulted in a planozygote with two longitudinal flagella. Hypnozygotes had a mandatory dormancy period of ca. 70 days. Germination resulted in planomeiocytes with two longitudinal flagella. Protargol-stained specimens indicate that nuclear cyclosis may occur in the planomeiocyte. Mis-identification of morphologically distinct life history stages and incomplete examination of thecal plate morphology in field specimens of *P. steidingerae* have led to taxonomic confusion.

O.14-01
Physiological stress responses of *Daphnia magna* to cyanobacteria and cyanobacterial compounds

Session: O.14 - Food Chains
Presentation time: 09:10 - 09:30

C Wiegand

Leibniz Institute of Freshwater Ecology, BERLIN, Germany

Cyanobacteria frequently flourish in eutrophic aquatic systems and several species are capable of producing toxic secondary metabolites, such as microcystins which specifically inhibit serine/threonine protein phosphatases, and cyanopeptolines and micropeptins leading to inhibition of trypsin, and chymotrypsin. Inhibition of grazing seems to be one of the ecological functions of the cyanobacterial bioactive compounds. With the help of several mechanisms, the grazing zooplankter *Daphnia magna* endures living in water bodies inhabited up to moderate densities of cyanobacteria. Amongst these is the detoxication of microcystins by conjugation to glutathione via the glutathione-S transferases. Furthermore, oxidative stress, caused by cyanobacterial compounds is reduced due to the activities of antioxidative enzymes in *D. magna* (superoxide dismutase, catalase, and glutathione peroxidase) showing different time kinetics of responses, depending on the application of either pure microcystin-LR or cyanobacterial crude extract. Changes in the total protein pattern were revealed using 2-D gel electrophoresis, and proteins were analyzed by MALDI-TOF/TOF mass spectrometry and identified via peptide mass fingerprint and additional MS/MS measurements. Enhanced induction of proteins involved in oxygen transport and oxidative metabolism were observed after exposure to cyanobacterial crude extract, whereas microcystin-LR caused a down regulation of protein expression.

O.14-02
Accumulation and transfer of the amnesic shellfish poisoning toxin domoic acid in the marine food webs off the Portuguese coast

Session: O.14 - Food Chains
Presentation time: 09:30 - 09:50

Pedro Costa[1], Susana Garrido[1], Rui Rosa[1], Marina Sequeira[2], Vanda Brotas[3], Maria Antonio Sampayo[1]
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[3]Instituto de Oceanografia, LISBOA, Portugal

While in North America hundreds of marine mammals and sea birds died after ingestion of fish contaminated with domoic acid (DA), in European coastal waters such outbreaks have not been described and transfer of DA through marine food webs has been little studied. With this work we aimed to study accumulation and transfer of DA along the marine food webs off the Portuguese coast. During 2001-2005 both pelagic and benthic members of the marine fauna were collected for DA determination. The toxin was detected in two species of crustaceans (*Polybius henslowii* and *Necora puber*), sardines (*Sardina pilchardus*) and six species of cephalopods (*Octopus vulgaris*, *Eledone moschata*, *E. cirrhosa*, *Sepia officinalis*, *S. elegans* and *S. orbignyana*). Since the diet of the common dolphin (*Delphinus delphis*) along the Portuguese coast is primarily dominated by sardines and cephalopods, tissue samples (kidney and intestine) of stranded marine mammals were used for DA detection. However, the analysed samples did not show contamination of the top of the marine food web. We present data showing DA produced by *Pseudo-nitzschia australis* that from interactions in the marine food web reaches several groups of animals with potential risks to ecosystem stability and human health safety.

O.14-03
Grazing, prey selectivity and toxin content of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa* feeding on *Dinophysis* spp. assemblages

Session: O.14 - Food Chains
Presentation time: 09:50 - 10:10

SK Sopanen[1], OS Setälä[1], RM Autio[2], K Erler[3]

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[2]Finnish Institute of Marine Research, HELSINKI, Finland
[3]Institute of Nutrition, University of Jena, JENA, Germany

Dinophysis acuminata, *D. norvegica* and *D. rotundata* are commonly found in the late summer plankton communities of the northern Baltic Sea. *Dinophysis*-derived toxins have previously been found in benthic organisms, but no information on toxin transport via the planktonic food webs exists. To investigate the role of copepods as grazers of *Dinophysis* spp. a series of experiments were carried out at the SW coast of Finland.
Grazing activity and prey selectivity of *Eurytemora affinis* and *Acartia bifilosa* were experimentally studied with field collected, concentrated plankton assemblages containing *Dinophysis* spp. offered as food. The experimental water was manipulated to establish both species diverse, and *Dinophysis* spp.– dominated microplankton assemblages. The copepod ingestion rates and selectivity in each experiment were estimated. Toxin content of copepods used in the incubations and field collected zooplankton were analysed.
The overall ingestion rates in *Dinophysis* - dominated units were 30.5 – 130 cells ind^{-1} d^{-1}. When food availability increased, other organisms were preferred, and the ingestion rates on *Dinophysis* spp. were more variable (0.9 – 149 cells ind^{-1} d^{-1}).
PTX-2 (142 pg ind^{-1}) was found in *E. affinis* after 24h incubation. Moreover, traces of PTX-2 and its seco acid were found from field collected zooplankton samples from the study area.

O.14-04
Karlotoxins mediate interactions between the mixotrophic dinoflagellate, *Karlodinium veneficum*, its prey, and its predators

Session: O.14 - Food Chains
Presentation time: 10:10 - 10:30

JE Adolf, D Krupatkina, TR Bachvaroff, AR Place

UMBI Center of Marine Biotechnology, BALTIMORE, United States of America

Little is known about the ecological mechanisms that allow the toxic dinoflagellate, *K. veneficum*, to bloom. Here, we tested hypotheses that the cytotoxic and ichthyotoxic karlotoxins produced by *K. veneficum* (1) allow greater feeding by *K. veneficum* on cryptophyte prey, *Storeatula major*, and (2) have anti-grazing properties against a common predator of *K. veneficum*, the heterotrophic dinoflagellate *Oxyrrhis marina*. Of 14 strains of *K. veneficum* offered *S. major* as prey only the non-toxic strains, MD5 and '*G. corsicum*', did not feed. *Oxyrrhis marina* feeding rate on *K. veneficum* was reduced significantly when fed toxic compared to non-toxic strains or when a sub-lethal dose of purified toxin was added to a non-toxic prey culture. The feeding rate of *O. marina* on non-toxic *K. veneficum* was reduced in mixed cultures containing both toxic and non-toxic strains. We suggest karlotoxin is part of an ecological strategy that increases K. veneficum cellular growth rate via mixotrophy while minimizing grazing losses. These mechanisms will work together to optimize population growth rates, increasing the likelihood of bloom formation. Further experiments are being conducted to test the ability of *K. veneficum* to compete with *O.*

marina for cryptophyte prey in mixed culture.

PL.06
Perilous neglect, reasoned accommodation, or transformation? Coping with changing species concepts in microeukaryotes

Session: PL..06 - Plenary VI - Taxonomy
Presentation time: 11:00 - 11:35

DG Mann, KM Evans

Royal Botanic Garden Edinburgh, EDINBURGH, United Kingdom

In sexual organisms where visual cues are important either for mating itself or to attract a mating vector, there are obvious, good reasons why we can construct a meaningful species-level taxonomy on the basis of morphological characters. Microalgae and protists are not all sexual and they do not see each other, and so any demand that species should be identifiable morphologically is clearly anthropocentric. The question is whether this demand is also unreasonable and counter-productive, given the functions of taxonomy to (1) categorize the living world for information storage, (2) facilitate communication, and (3) allow prediction. Recent molecular and mating data indicate that cryptic/semicryptic species are widespread in microscopic eukaryotes; the relationship between morphological variation and phylogeny is weak; complexes of semicryptic species can be several millions of years old (allowing their members to become widely distributed); and significant local and temporal differentiation of populations can develop over relatively short time-scales. Mating systems can vary significantly among close relatives, probably producing complex variation patterns similar to those in multicellular organisms. Close relatives are not always the most similar ecologically. Overall, then, a taxonomy that ignores cryptic microeukaryote species will significantly degrade dependent science. Molecular 'bar-coding' is becoming an essential underpinning.

O.15-01
Characterization of NW Mediterranean *Karlodinium* spp. (Dinophyceae) strains using morphological, molecular, chemical and physiological methodologies

Session: O.15 - Taxonomy
Presentation time: 11:40 - 12:00

E Garcés[1], M Fernandez[2],A Penna[3], K van Lenning[4], A Gutierrez[4]
M Zapata[5]

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Recurrent fish kills in the Spanish Alfacs Bay (NW Mediterranean) have been detected during winter seasons since 1994, and were attributed to an unarmoured, ichthyotoxic, dinoflagellate, initially identified as *Gyrodinium corsicum*. Several strains were isolated from the Bay and their clonal cultures were compared by combined techniques, including light- and electron microscopy, Internal Transcribed Spacer & 5.8S rDNA nucleotide sequencing and HPLC pigment analyses, together with studies of their photochemical

performance, growth rates and toxicity. Using phylogenetic analyses all strains were identified as members of the genus *Karlodinium*, but they were separated in two genetically different groups. These groups, identified as *Karlodinium veneficum* and *K. armiger*, were also supported by the other techniques employed. Specific differences in pigment patterns coincided with that expected for low (*K. veneficum*) and high-light (*K. armiger*) adapted relatives. The higher photosynthetic efficiency of *K. veneficum* and the longer reactivation times of the PS II reaction centers observed for *Karlodinium armiger* were in agreement with this hypothesis. The two species differed in toxicity, but strains employed always induced mortality when incubated with bivalves, rotifers and finfish. Compare to *K. armiger* strains of *K. veneficum* yielded higher cell densities, but with lower growth rates.

O.15-02
Potential harmful cyanobacteria in drinking water reservoirs of Ho Chi Minh City, Vietnam - toxicity and molecular phylogeny

Session: O.15 - Taxonomy
Presentation time: 12:00 - 12:20

S Christensen[1], N Daugbjerg[1], Ø Moestrup[1], H Annadotter[2], G Cronberg[2]
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[2]Institute of Ecology, LUND, Sweden

Three large freshwater reservoirs are the primary water supply for approximately 8 million people of Ho Chi Minh City, Vietnam. There have been reports of cyanobacterial blooms in these reservoirs, but no survey of the occurrence of cyanobacterial toxins in the reservoirs and in the drinking water has previously been made. In this study, a survey of the occurrence of cyanobacteria and the cyanobacterial toxin microcystin was conducted in two of the three reservoirs. The tap water was likewise tested for the presence of microcystin. Twelve cultures of filamentous cyanobacteria representing the orders Oscillatoriales and Nostocales were established, described, photographed and tested for microcystin production. Their phylogenetic relationship was investigated on the basis of phycocyanin gene sequence analyses. Cultures of *Microcystis* were established and tested for microcystin production. Microcystin concentrations were measured by ELISA and HPLC. Toxic and potentially toxic species were observed in both reservoirs. Microcystin concentrations were below the WHO guideline value of 1.0 µg/l, but tap water concentrations corresponded to the concentrations in the reservoirs. All investigated genera within Oscillatoriales and Nostocales were polyphyletic according to the phycocyanin sequences. Phycocyanin sequence similarities of 100% indicated a recent lateral gene transfer between species of *Arthrospira* and *Oscillatoria*.

O.15-03
Genetic and phenotypic differences among species and strains of potential fish-killing raphidophytes in the Mediterranean

Session: O.15 - Taxonomy
Presentation time: 12:20 - 12:40

S Kloepper[1], U John[1], U Tillmann[1], A Zingone[2], AD Cembella[1]

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Dense raphidophyte blooms are a cause of mass fish mortalities in coastal areas throughout the world. The recent dramatic global growth of aquaculture, especially fish farming, has led to increased requirement for knowledge on characterization and toxigenicity of raphidophyte blooms. In the Mediterranean, raphidophytes form annual blooms in coastal waters, thereby posing a threat to fish aquaculture. We investigated a mixed bloom event involving the raphidophytes *Chattonella* sp. and *Fibrocapsa* sp. on the Adriatic coast of Italy, including molecular genetic, morphological and ecological aspects. Light and electron microscopy revealed one of the species to be *Chattonella subsalsa* as described by Biecheler (1936). Nevertheless, genetic markers for nuclear and ribosomal DNA (LSU, SSU, ITS, psaA and RubisCo) differentiated this *Chattonella*, and with less distinctness, *Fibrocapsa* sp., from other known strains of the respective genera. Phenotypic differences among isolated clones were determined from profiles of polyunsaturated fatty acids (PUFAs) and activity of reactive oxygen species (ROS), which are putatively responsible for ichthyotoxicity. Strains were bioassayed with a fish erythrocyte lytic assay and the brine shrimp *Artemia salina* test. The relationship (if any) between toxicity and phenotypic and genotypic characteristics is complex and not yet resolved.

O.15-04
How many different species are in the *Alexandrium tamarense* /catenella / fundyense complex?

Session: O.15 - Taxonomy
Presentation time: 12:40 - 13:00

S Fraga[1], RI Figueroa[1], I Bravo[1], N Sampedro[2], JM Franco[3], A Penna[4], I Ramilo[1], A Fernández-Villamarín[1]

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[4]University of Urbino, PESARO, Italy

The *Alexandrium tamarense /catenella / fundyense* species complex comprises four genetically different clades in which the similarity between strains is larger in geographically related species than between geographically distant strains of the same species. These clades are the North Atlantic, the Western European, the Temperate Asian and the Mediterranean clades. The morphological characters currently used to differentiate the species within this complex are the presence or absence of a ventral pore in 1' plate, the ability to form chains, and the relation between the transdiameter and the length of the cells. In *Alexandrium minutum* the presence of the ventral pore is not a constant character, and in other species the ability to form longer or shorter chains is strain dependent, and the relation between the transdiameter and length of cells depends on whether or not they form chains. These characters alone are no longer valid to differentiate among species of this complex. In our opinion the four clades correspond to four different cryptic species, and new morphological criteria are necessary to define them. We present data to support this hypothesis based on different growth rates in relation to temperature, mating experiments, toxin profiles and morphology.

O.16-01
A domoic acid immunosensor onboard the Environmental Sample Processor: the first steps toward remote, sub-surface phycotoxin detection

Session: O.16 - Monitoring 1
Presentation time: 11:40 - 12:00

GJ Doucette[1], CA Scholin[2], CM Mikulski[1], R Marin III[2], S Jensen[2], B Roman[2], D Greenfield[2], KL King[1], J Feldman[3], G Massion[2], CT Elliott[4]

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The emergence of ocean observing initiatives highlights the benefits of developing remote, in-situ diagnostic capabilities for biological/biochemical constituents, including harmful algal bloom (HAB) taxa, their genes, gene products, and metabolites. The ability to detect such targets, especially HAB species and toxins, in real or near-real time is of immediate utility to researchers studying HAB/toxin dynamics and to coastal managers charged with mitigating the socioeconomic impacts of blooms. The Environmental Sample Processor (ESP; www.mbari.org/microbial/ESP) was developed for the autonomous, sub-surface application of molecular diagnostic tests and during initial deployments has successfully run DNA probe arrays for several HAB species. To address the need for concurrent detection of algal toxins, which are known to vary widely in concentration depending on algal physiological status, we recently fielded a toxin extraction protocol coupled with immuno-based toxin arrays during deployments of a second generation ESP. Through application of species and toxin arrays, known toxic *Pseudo-nitzschia* spp. and domoic acid (DA) were detected concurrently onboard the ESP in Monterey Bay, CA, USA, representing the first remote, integrated assessment of algal cell abundance and toxin concentration in coastal waters. Refined calibration of the DA assay will yield more accurate estimates of a bloom's toxicity and potential impacts.

O.16-02
The detection of toxic algae by a new developed rRNA biosensor (EU-Project ALGADEC)

Session: O.16 - Monitoring 1
Presentation time: 12:00 - 12:20

S Diercks, K Metfies, LK Medlin

Alfred Wegener Institute, BREMERHAVEN, Germany

The EU-project ALGADEC aims to develop a hand-held biosensor for *in situ* analysis of toxic algae which can serve as an early warning system to prevent toxication of animals and consumers. Identification of toxic algae is based on molecular probes that specifically target its rRNA. Taxon- specific probes were developed for HAB algae that occur in three different coastal areas in Europe. A sandwich-hybridization assay is used to detect the rRNA. A capture probe, immobilised on the biosensor, binds to RNA-strands isolated from the target organism, then a digoxigen-labelled second probe binds. An antibody-enzyme complex directed against digoxigenin is applied onto the sensor. Substrate is added and a redox-reaction takes place. The resulting electric current is measured and the amount of bound rRNA is proportional to the electric current. The adaptation to the sensor and the tests for specificity of probes were done by using laboratory strains. Validation of probe signals are carried out against total rRNA over the growth cycle of the algae and under different environmental conditions. Finally sensors will be tested in order to obtain a calibration curve that will allow for conversion of the electronic signal into concentration of toxic cells with special software.

O.16-03
Identifying and detecting harmful algal bloom species using a colour imaging flow cytometer (FlowCAM®)

Session: O.16 - Monitoring 1
Presentation time: 12:20 - 12:40

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The ability to detect, identify and enumerate harmful algal species is a requirement in coastal ecosystems for monitoring programs and early detection of harmful bloom events. To date, most monitoring programs utilize microscopes for identifying bloom species in a laboratory or from field samples, which can be laborious and time consuming. Recently, however, automated techniques for monitoring and detecting target species have been tested in phytoplankton monitoring programs. These methods include instruments that analyze the molecular DNA/RNA content within field samples, examine optical properties of the water (such as pigment composition), and utilize optical flow through systems. FlowCAM is an imaging-flow-cytometer that combines the capabilities of a flow cytometer with a digital-imaging microscope and automates phytoplankton detection and enumeration. Previously, FlowCAM has been shown to successfully detect and enumerate harmful algal bloom species (*Alexandrium fundyense* and *Karenia brevis*) from both laboratory and field samples. Here we present data from a new 'color' FlowCAM. The use of color provides additional criteria for distinguishing between closely related harmful algal species. The key benefits of this technology are the ability to analyze phytoplankton continuously, determine the size, and most importantly the collection of color digital images for further analysis.

O.16-04
Quantitative Real-time PCR detection of harmful algae

Session: O.16 - Monitoring 1
Presentation time: 12:40 - 13:00

MF de Salas, CJS Bolch
University of Tasmania, HOBART, TAS, Australia

A range of species-specific qPCR assays were developed to enumerate low concentrations of difficult to identify harmful marine dinoflagellates in the genera *Alexandrium*, *Karenia*, *Karlodinium* and *Takayama*. These assays used species-specific primers together with species specific dual-labelled fluorogenic probes for maximum sensitivity and specificity. Detection levels of one single cell could be consistently achieved using this technology, significantly reducing the need for skilled microscopy in routing monitoring. This was combined with the use of a crude lysate for optimal quantitation to produce a result that can be directly checked against existing action-level tables for target species. *Alexandrium* probes were also successful in detecting hypnozygotes, extending the rage of possible applications to routine sediment surveys and ballast water monitoring.

PL.07
Diatom genomics: new insights into diatom toxicity

Session: PL..07 - Plenary VII - Genomics
Presentation time: 08:30 - 09:05

Virginia Armbrust

University of Washington, SEATTLE, United States of America

Whole genome sequences for two diatoms, *Thalassiosira pseudonana* (centric diatom) and *Phaeodactylum tricornutum* (pennate diatom) have been completed and are (or soon will be) publicly available. Whole genome sequencing of the toxigenic diatom *Pseudo-nitzschia multiseries* is now underway and is scheduled to be completed in 2007. Under still poorly defined environmental conditions, *Pseudo-nitzschia* species can produce the neurotoxin domoic acid, which is the causative agent of amnesic shellfish poisoning. The growing data base of DNA sequence information allows new approaches to understand specific aspects of diatom physiology such as toxin production. For example, comparative and whole genome transcriptional analyses are being used to define features that appear common to diatoms in general and specific to pennate or centric diatoms in particular. In addition, in advance of the availability of the whole genome sequence information for *P. multiseries*, we are using subtractive hybridization approaches to identify genes specifically upregulated when *Pseudo-nitzschia* species are limited with silicate and induced to produce domoic acid. Ultimately, we will use this data base to ask questions about field populations of *Pseudo-nitzschia*. I will highlight ways in which genome data can be used to ask questions about different aspects of phytoplankton physiology and ecology.

O.17-01
Nutrient-regulated transcriptional changes in *Aureococcus anophagefferens* identified with long-SAGE (serial analysis of gene expression)

Session: O.17 - Genomics
Presentation time: 09:10 - 09:30

ST Dyhrman, ST Haley, LL Wurch, ED Orchard

Woods Hole Oceanographic Institution, WOODS HOLE, United States of America

Nutrient availability can influence important aspects of harmful algae biology and ecology, such as growth, toxin production, and life cycle transformations. Despite the importance of nutritional physiology to these processes, fundamental gaps remain in our understanding of nutrient-scavenging mechanisms in harmful species. Using long-serial analysis of gene expression (Long-

SAGE) we have examined transcriptional patterns in three *Aureococcus anophagefferens* libraries, nutrient-replete, phosphorus-starved (-P), and nitrogen-starved (-N), designed to identify nutrient stress responses. Long-SAGE examines gene expression patterns without a priori knowledge of gene sequences via the detection of 21bp sequence tags. To date we have sampled over 75,000 *A. anophagefferens* sequence tags. Ongoing analyses suggest that *A. anophagefferens* has a strong transcriptional response to nutrient starvation, with 71 sequence tags significantly (R=2) up-regulated in the –N library, and 179 sequence tags (R=2) up-regulated in the –P library. Unlike the genome, which is essentially static, these patterns of gene expression are modulated by the nutritional physiology of the cell and the ongoing annotation of these sequence tags will provide a dynamic link between *A. anophagefferens* and its cellular functioning in coastal systems.

O.17-02
EST-based gene discovery and expression analysis in Alexandrium.

Session: O.17 - Genomics
Presentation time: 09:30 - 09:50

JD Hackett, DM Anderson

Woods Hole Oceanographic Institution, WOODS HOLE, United States of America

Expressed sequence tag (EST)-based approaches are an important tool for facilitating gene discovery for organisms without a complete genome sequence. We used a highly efficient strategy using normalized and subtracted cDNA libraries to generate ESTs for the toxic dinoflagellate *Alexandrium tamarense* (9,000 unique ESTs). We are now using these data to determine the metabolic capabilities of *Alexandrium*. The ESTs were analyzed to determine the metabolic pathways present in Alexandrium and to identify genes that may be involved in saxitoxin synthesis. We are also using quantitative-PCR to analyze gene expression under nitrogen and phosphorus limitation using cultured strains. Genes that show regulation under these nutrient stress conditions are tested on samples collected from a natural toxic bloom of *Alexandrium*. The objective of this work is to design a gene expression 'tool kit' that can be used to determine the expression of genes involved in nutrient utilization from natural *Alexandrium* blooms.

O.17-03
Identification of cellular stress and death-associated genes in *Karenia brevis* as potential biomarkers for bloom termination

Session: O.17 - Genomics
Presentation time: 09:50 - 10:10

FM van Dolah, KB Lidie, JS Morey, EA Monroe, JC Ryan

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Karenia brevis is responsible for brevetoxin-producing red tides in the Gulf of Mexico. Current HAB forecasting tools are capable of projecting the movement of *K. brevis* blooms towards vulnerable communities; however, lack of insight into mechanisms controlling cell death in dinoflagellates makes forecasting of bloom termination currently unfeasible. In this study therefore we sought to identify genes in *K. brevis* expressed under conditions leading to cell death. An 11,000 gene *K. brevis* DNA microarray was used to assess global transcript profiles under a variety of acute (heat shock, peroxide, lead, NaNO2, and paraquot) and chronic stress (N or P depletion, dark, and cell senescence). We identified a suite of genes up-regulated under all conditions that result in decreased viability and another suite consistently down-regulated. Up-regulated genes include members of the ubiquitin pathway, senescence-associated cys proteases, and calpains. Genes involved in translation and photosynthesis are consistently down-regulated. Components of the programmed cell death pathway are present (metacaspase, caspase recruitment domain proteins, death-associated kinase), but do not respond at the transcriptional level. The expression of this gene set is currently under investigation in field populations of *K. brevis* of known growth/stress status to assess their potential for biomarkers of bloom termination.

O.17-04
A genomic approach towards a better understanding of domoic acid production in the marine diatom *Pseudo-nitzschia multistriata*

Session: O.17 - Genomics
Presentation time: 10:10 - 10:30

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Within the diatoms the genus *Pseudo-nitzschia* has gained lively interest since 1987 when P. multiseries caused an Amnesic Shellfish Poisoning (ASP) event along Prince Edward Island, Canada. Production of domoic acid seems to be tightly controlled by the diatom and several enzymes that might be involved in the metabolic pathway were recently suggested. Nevertheless, the key enzyme system facilitating the fusion and transformation of the two putative precursors geranylpyrophosphate and 3-Hydroxy-glutamic acid still remain unknown. In a first step we determined the physiological limits of *P. multistriata* to temperature, salinity and nutrient limitation. Thereafter, we investigate toxin production under those physiological conditions that stimulate production of domoic acid. In parallel gene expression of approx. 4500 genes is measured. Therefore, we use a microarray that is based on the EST-sequencing of a normalised cDNA library of *P. multistriata*. This approach enables us to make an in silico subtraction of expressed genes in relation to toxin production resulting in a set of candidate genes putatively involved in toxin synthesis.

O.18-01
Inter-annual variability of *Alexandrium* blooms in Cork Harbour, Ireland

Session: O.18 - Ecology & Oceanography 1
Presentation time: 09:10 - 09:30

A Ní Rathaille, N Touzet, R Raine
Martin Ryan Institute, GALWAY, Ireland

Blooms of *Alexandrium* are a recurring problem in the retentive North Channel of Cork Harbour on the south coast of Ireland. Annual variations of these blooms include the timing of their initiation, their intensity and their duration. They can often lead to toxic events and shellfish closures in the North

Channel area. Field data from the 2004 and 2005 bloom seasons are presented. These data sets provide evidence to show that the inter-annual variability of these blooms is directly related to the physical regime, namely temperature and light levels within the water column and tidal dilution. Results of laboratory experiments investigating the effects of both temperature and light on the growth rates of *A. minutum* and *A. tamarense*, species that co-exist within the North Channel, are presented. The control by tidal dilution in the North Channel varies substantially between spring and neap tides. It is the balance between the maximum growth rates, as determined by temperature and light, and the tidal dilution, as determined by the time of year, that dictate the initiation, intensity and duration of the observed blooms.

O.18-02
Eutrophication and HABs- a global change perspective

Session: O.18 - Ecology & Oceanography 1
Presentation time: 09:30 - 09:50

PM Glibert[1], S Seitzinger[2], RW Howarth[3], JM Burkholder[4]

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Eutrophication-related HABs are growing in frequency, duration and toxic impacts in many parts of the world. The past several decades have witnessed a dramatic increase in the availability of nutrients on land, in the atmosphere and in the ocean. This increase is the result of rapid growth in world population, and in the use of synthetic fertilizers, the development of concentrated animal and aquaculture operations, and the combustion of fossil fuels. This increase is especially apparent for nitrogen, as rates of application of nitrogen fertilizers have increased much faster than those of phosphorus, and has led to an accelerated nitrogen cycle globally. Although there are multiple reasons for the global expansion in HABs, and relationships between eutrophication and HAB proliferation are complex and not equally applicable to all species, the patterns of proliferation of many HAB species mirror the increase in application on, and export of, nitrogen from many regions of the world. Of increasing concern are the projections that 1) human population growth will be disproportionately in many coastal regions, and 2) global climate changes will lead to increased precipitation in many of these nitrogen-rich regions, increasing nitrogen export and thus the potential for further HAB blooms.

O.18-03
Thin layers of *Pseudo-nitzschia* spp and the fate of *Dinophysis acuminata* during an upwelling-downwelling cycle in a Galician ria

Session: O.18 - Ecology & Oceanography 1
Presentation time: 09:50 - 10:10

B Reguera[1], L Velo[1], S González-Gil[1], P Gentien[2], M Lunven[2], C Bechemin[2], L Fernand[3], R Raine[4]

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Measurements of the physical and biological environment were carried out in the Ria de Pontevedra (Galician Rías Baixas, Spain) over a two-week period in June 2005. Fine-scale vertical distributions of phytoplankton and shear were obtained using the IFREMER Particle Profiler and Fine Scale Sampler, coupled with measurements from a Nortek acoustic Doppler velocimeter, *in situ* observations of live samples (autofluorescence) and stained dinoflagellates. A sequence of upwelling-relaxation-upwelling-downwelling events was observed. Thin layers of *Pseudo-nitzschia* spp. and other diatoms (up to 30 µg chlorophyll a · L^{-1}) developed and persisted in the pycnocline region, above the cooler (12.5 ºC) nutrient-rich (>10µM nitrate) upwelled water, but were vertically displaced and eroded during downwelling. A population of *D. acuminata*, that had been evident since early March, was never found within the thin layers but instead was confined to the warmer surface (0-4m) layers throughout the entire survey. These cells did not perform any vertical migration. The results showed the need to define sub-surface thin layer characteristics suitable for individual species. In addition, the importance of the phase of the population growth to determine physical-biological interactions and behaviour of *Dinophysis* in relation to thin layers is highlighted.

O.18-04
Positive feedback and the development and persistence of ecosystem disruptive algal blooms

Session: O.18 - Ecology & Oceanography 1
Presentation time: 10:10 - 10:30

WG Sunda, DR Hardison
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Harmful algal blooms (HABs) have occurred with increasing frequency in recent years with eutrophication and other anthropogenic alterations of coastal ecosystems. Many of these blooms severely disrupt ecosystem function, and can be referred to as ecosystem disruptive algal blooms (EDABs). These blooms are typically caused by toxic or unpalatable species that decrease grazing rates by herbivores, and thereby disrupt transfer of nutrients and energy to higher trophic levels, and decrease nutrient recycling. Many factors, such as nutrient availability and herbivore grazing have been proposed to separately influence EDAB dynamics, but interactions among these factors have rarely been considered. Here we describe positive feedback interactions among nutrient availability, herbivore grazing, and nutrient cycling, which can to substantially influence the dynamics of EDAB events. The positive feedbacks result from reduced grazing rates on EDAB species, which promote the proliferation of these algae and decrease grazer-mediated recycling of nutrients. These effects in turn decrease nutrient availability. Since many EDAB species are well-adapted to nutrient-stressed environments and many exhibit increased toxin production and toxicity under nutrient limitation, positive feedbacks are established which can greatly increase the rate of bloom development, and promote bloom persistence and adverse effects on the ecosystem.

O.19-01
Field applications for remote detection of harmful algae using the Environmental Sample Processor: Spring-Summer 2006

Session: O.19 - Monitoring 2
Presentation time: 11:00 - 11:20

Dianne I Greenfield[1], CA Scholin[1], S Jensen[1], R III Marin[1], B Roman[1], B Massion[1], GJ Doucette[2]

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Molecular approaches for identifying harmful algal bloom (HAB) species and affiliated toxins are central to research and monitoring, but such methods require the return of discrete samples for laboratory analysis. This impediment is overcome with the Environmental Sample Processor (ESP), an instrument that detects remotely, subsurface, and in near real-time, a wide range of microorganisms and substances they produce (http://www.mbari.org/microbial/ESP). The first-generation ESP verified basic concepts, such as sample archive and DNA array processing. The second-generation (2G ESP), a comparatively smaller, faster, and more robust version, was been deployed for the first time in Monterey Bay, CA (USA) during spring-summer of 2006 for ~20d per deployment. During this field season, the 2G ESP successfully automated detection of a number of harmful species, including diatoms of the genus *Pseudo-nitzschia*, some of which produce the toxin domoic acid. In addition to in situ detection, we attempted to ground truth instrument data by period water sampling and analyses using laboratory versions of molecular assays that are emulated within the ESP. Here we present our field findings to date, including species detected remotely during 2006, domoic acid (Doucette *et al*.), environmental data, and our capability to ground-truth instrument data.

O.19-02
Monitoring of lipophilic shellfish toxins using SPATT (Solid Phase Adsorption Toxin Tracking) in Nova Scotia, Canada

Session: O.19 - Monitoring 2
Presentation time: 11:20 - 11:40

CM Garnett[1], CM Rafuse[1], NI Lewis[1], S Kirchhoff[2], J Cullen[2], MA Quilliam[1]

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Field studies were undertaken in Ship Harbour and Lunenburg Bay, Nova Scotia, Canada, from May to November, 2005. Solid Phase Adsorption Toxin Tracking (SPATT) bags were deployed weekly in conjunction with a variety of physico-chemical measurements including temperature, conductivity and light attenuation. Sampling of phytoplankton was conducted at both sites and blue mussels (*Mytilis edulis*) were collected at the Ship Harbour site. SPATT extracts were analysed by LC-MS/MS using a multi-toxin analysis for lipophilic toxins. The following toxins were detected: spirolides, dinophysistoxin-1, pectenotoxin-2, pectenotoxin-2 seco acid, yessotoxin and azaspiracids. The concentration effect of toxins from the water column by the SPATT bags was supported by evidence of toxins in the SPATT extracts when no toxins were detected in planktonic net tow samples. In addition, changes in the toxin

profiles over the sampling period at both sites were observed and these were related to changes in the dinoflagellate community structure. These results are compared to SPATT and phytoplankton data obtained from Ship Harbour during 2004. Correlation between the concentration of shellfish toxins in the SPATT extracts and mussel tissue was examined to evaluate the application of this technique as an early warning technology for the aquaculture industry.

O.19-03
Developing operational capabilities for nowcasts and forecasts of harmful algal blooms

Session: O.19 - Monitoring 2
Presentation time: 11:40 - 12:00

RP Stumpf, MC Tomlinson`

NOAA National Ocean Service, SILVER SPRING, MD, United States of America

Managers and communities need forecasting systems that address 'nowcasts' — where a harmful algal bloom (HAB) is today; and 'forecasts' — where it will be in the near future. They often want to know if a HAB will initiate or dissipate. While characteristics of HABs differ, and regional systems are necessary, there are commonalities between forecasting systems.
Forecasting systems have three basic requirements: data on HAB locations; transport models that address management requirements; and analysis of the data and models that make sense to managers. To determine HAB location, the suite of observations may include water samples, manual or automated detectors, and remote observations. To determine transport, the models can include heuristic models and 1-D, 2-D, or 3-D transport or circulation models. The effective integration of observations and models requires an analyst, much as an analyst is needed for a weather forecast.
Predicting initiation and dissipation may use different ecological models and observational schemes. These will tend to involve a greater diversity of physical and ecological observations. While an initiation model can aid in directing sampling to find a new HAB, the requirements of that model should not be confused with the basic 'nowcast' requirements of location, transport, and analysis.

O.19-04
Early warning of cyanobacteria in water reservoirs

Session: O.19 - Monitoring 2
Presentation time: 12:00 - 12:20

KG Garde[1], T Jurczak[2], K Izydorczyk[3], L Schlüter[4], H Kaas[1]

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Mass occurrences of toxic cyanobacteria pose a serious threat for water works abstracting water from surface waters around the world. If cyanotoxins enter the distribution net, they may cause illnesses in humans and in case of long-term exposure cancer risk hazards. Thus early warning methodologies are called for, making water works able to detect cyanobacterial problems in due time to activate proper measures. A method increasingly used is *in situ* multichannel fluorometry enabling quantitative estimations of

occurrences of algal groups; including cyanobacteria. In the present study the compliance between this strategy and other methods commonly applied to describe and quantify phytoplankton composition were compared; including lab fluorometry, spectrophotometry and HPLC pigment analyses and microscopically examination. In addition, the occurrence of microcystins was assessed. In general, acceptable coherence was observed between the different strategies when accessed based on the occurrence and dominance of cyanobacteria. Regarding the other algal groups obvious discrepancies occurred; i.e. the *in situ* fluorometer method was not able to describe the phytoplankton community properly.

O.19-05
Phytoplankton community composition observed by autonomous underwater vehicle

Session: O.19 - Monitoring 2
Presentation time: 12:20 - 12:40

GJ Kirkpatrick[1], MA Moline[2], SE Lohrenz[3], OM Schofield[4]

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Laboratory and field studies have demonstrated the feasibility of detecting *Karenia brevis* blooms in the eastern Gulf of Mexico utilizing light absorbance spectra. Development of this technique has been aimed at providing more timely access to data and information on the initiation, transport, and effects of *K. brevis* blooms. Management efforts to mitigate the harmful effects of blooms will require temporal and spatial monitoring of phytoplankton community taxonomic composition and dynamics. To achieve this taxonomic discrimination, laboratory cultures of 12 species of microalgae representing five taxonomic classes were used to develop a library of target classes. A fitting routine involving multiple least-squares analyses was applied to BreveBuster absorbance spectra to determine the 'best fit' estimates of chlorophyll a concentration contributed by each class in both laboratory culture mixes and natural mixed populations. A ten-day deployment of a BreveBuster on an autonomous underwater vehicle (AUV) off the west coast of Florida in September and October 2004 detected a *Karenia brevis* population associated with cyanobacteria and diatom populations which had not been observed by manual sampling. Multiple transects across the shelf by the AUV over this ten-day period illustrated the spatial and temporal dynamics of the phytoplankton community.

O.19-06
Retrospective GIS analyses of the Florida red tide database

Session: O.19 - Monitoring 2
Presentation time: 12:40 - 13:00

KA Steidinger[1], JA Tustison[2], CA Heil[2]

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[2]FL Fish & Wildlife Conservation Comm., ST. PETERSBURG, United States of America

Florida red tides (*Karenia brevis*) are the most frequent and longest

lasting marine HABs in the world. Red tide data visualization using layered Geographic Information System (GIS) datasets allows display of K. brevis count data over a 50 year period. The database contains 'event response' results as well as cruise and transect data with fixed sampling stations collected at least monthly. When data on count, location, depth, date, and time are displayed on a daily basis, a sequential display of data keyed by symbols and colour shows the progression of a bloom being transported cross-shelf and long-shore. This data layer can be integrated with winds, current models, hurricanes and other variables to visualize animated movement and forcing functions on the west Florida shelf. Although the database is composed of disparate datasets from haphazard, directed, or repetitive sampling, GIS technology offers geospatial analytical capabilities not available for the conventional database. The following can be visualized by specific red tide datasets: offshore initiation; transport from the Gulf of Mexico to the Florida east coast; long-shore movement, both south to north and vise versa; and the forcing of currents on initial southerly transport. In addition, GIS analyses allows for improvement of sampling design.

O.20-01
A harmful algal bloom occurrence in Barangay Kirayan Norte, Miagao, Iloilo, Philippines

Session: O.20 - Regional events
Presentation time: 11:00 - 11:20

JP Peralta[1], SS Garibay[1], JRN Noble[2], RMM Espina[3], AN Nualla[3]
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[2]Municipality of Miag-ao, ILOILO, Philippines
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On March 1, 2005, a dense mat of floating algae had accumulated inshore, and began to rot. Coastal residents of Barangay Kirayan Norte, Miagao, Iloilo, Philippines experienced problems sleeping at night, children had difficulty breathing, and some had skin irritations and itchiness. Since the sea breeze flows inland, the residents were affected by the rotting algae, which smelled seaweedy and ammoniacal.
One of the residents tried to mitigate the dilemma by harvesting the wash-up algae, which had piled inshore, and buried it in a sand pit. The person, who harvested the algae with his unprotected bare hands, experienced swelling of his face and other skin irritations.
The probable causative organism was identified as *Lyngbya majuscula*, a filamentous cyanobacterium whose colour is dark-green to black. The presence of *L. majuscula* in the coastal communities now poses risks to its residents. Monitoring and further study of this phenomenon is therefore highly recommended.
The paper reports on the occurrence of the harmful algal bloom, advocates the monitoring of the phenomenon, and reports initiatives being undertaken by U.P. Visayas.

O.20-02
Cochlodinium blooms in Sabah, Malaysia

Session: O.20 - Regional events
Presentation time: 11:20 - 11:40

A Anton[1], PL Teoh[1], S Mustaffa[1], L Nordin[2]

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Harmful algal blooms in Malaysia are mostly confined to the west coast of Sabah. Since it was first reported in 1976, *Pyrodinium bahamense* var. *compressum* has been the main causative organism, which has resulted in public health problems and diminution of shellfish cultures. In January 2005, blooms of *Cochlodinium polykrikoides* were first observed in the Sepanggar Bay off Kota Kinabalu, causing fish kills and economic losses to the aquaculture industry. Population studies of the blooms showed a perpectual pattern of occurrence in 2005, with high densities (> 104 cells l^{-1}) recurring around areas of aquaculture activities and replacing the dominant HAB species *Pyrodinium bahamense* var. *compressum*. The origin of these blooms has been speculated to be caused by circulation currents in the South China Sea and/or aquaculture activities of introduced species in the coastal waters off Kota Kinabalu. The 16S rDNA of *C. polykrikoides* isolated from waters around Kota Kinabalu has been sequenced and used for comparison with other existing sequences. A multidisciplinary approach to study *Cochlodinium* red tides, including ocean current patterns, water quality, and remote sensing is being undertaken, to further understand the factors causing the blooms for the purpose of management and mitigation.

O.20-03
Massive fish kills in the Philippines caused by *Cochlodinium* and *Prorocentrum*

Session: O.20 - Regional events
Presentation time: 11:40 - 12:00

RV Azanza

The Marine Science Institute, QUEZON CITY, Philippines

There have been few and scattered reports of fish kills in the Philippines. Not much attention has been given to these events until a *Prorocentrum minimum* bloom occurred for the first time in 2002, that coincided with mass mortality of cultured milkfish, thus wreaking havoc to the local fishermen's livelihood. This fish kill phenomenon, that happened in the municipality of Bolinao, Pangasinan, north of the Philippines, has been attributed to uncontrolled proliferation of fish cages and pens. Yearly harmful algal blooms of other species have been experienced in this area.

Palawan, Southwestern Philippines has a rich marine life, supplying 60% of Manila's fish requirements, and contributes to half of the national fish export. With a relatively more pristine water, Palawan experienced its major red tide outbreak in 2005. A *Cochlodinium polykrikoide*s bloom spanned 500km of Palawan's coastline and lasted for four months. Satellite images from MODIS Aqua Level 2 data revealed chlorophyll-a levels as high as >0.5 mg/m^3 from constructed mosaics of its monthly distribution.

This paper reports on the series of events that led to fish kills in two relatively different water systems. Factors that contributed to the blooms will be discussed and contrasted.

O.20-04
Alexandrium fundyense - red tides, PSP shellfish toxicity, salmon mortalities and human illnesses in 2003-04 –before and after

Session: O.20 - Regional events
Presentation time: 12:00 - 12:20

JL Martin[1,2], FH Page[1], MM LeGresley[1]
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[2]Fisheries and Oceans Canada, Canada

The Bay of Fundy has a long history of *Alexandrium fundyense* blooms and annual shellfish harvesting area closures, generally during summer months, due to unsafe levels of PSP toxins. In addition to shellfish closures, herring mortalities occurred in 1976 and 1979, the salmon farming industry suffered mortalities in 2003-04 and human illnesses have occurred through the years.
Data on PS toxins in shellfish have been collected since the 1940s and provide an important perspective on inter-annual and seasonal *A. fundyense* patterns. Data indicate that PSP has been present throughout much of the Bay of Fundy since the early 1940s. and there are consecutive years of greater toxicities.
Regular phytoplankton sampling since 1988 shows that *A. fundyense* cell concentrations are generally greatest in the offshore regions in close proximity to major cyst deposits and tend to be dispersed through the exposed regions through water movements and circulation. Highest cells densities since 1988 were observed in 2003 (8.8 x 105 cells•L-1) in the Grand Manan Island area and in 2004 in Bliss Harbour (> 3 million cells.L^{-1}) and resulted in salmon mortalities during both years. Patterns in shellfish toxicity prior to and following the red tide events are discussed.

O.20-05
Dynamics of blooms of cf *Chattonella verruculosa* in the Skagerrak and the Kattegat

Session: O.20 - Regional events
Presentation time: 12:20 - 12:40

B Karlson[1], E Almroth[1], P Andersen[2], K Eilola[1], M Kuylenstierna[3], L-J Naustvoll[4]
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[4]Flødevigen Marine Research Station, HIS, Norway

Large blooms of cf. *Chattonella verruculosa* were observed in the Skagerrak area for the first time in 1998. Blooms have occurred in the area also in years 2000, 2001 and 2006. An overview of the bloom events is presented. The organism may be an introduced species but reanalysis of preserved samples show that it was present in the area in 1993. It now seems to be an established species in the area. The blooms are described using observations from ship sampling (cell counts) and satellites. Also physical and biogeochemical models are used for describing the events. Models results indicate that low turbulence conditions may be an important factor for bloom initiation.

O.20-06
Role of short-term climate change on outbreak and succession of large scale HABs along east Chinese coast in 2005

Session: O.20 - Regional events

Presentation time: 12:40 - 13:00

Mingjiang Zhou[1], Mingyuan Zhu[2], Yunfeng Wang[1], Dedi Zhu[3], Songhui Lv[4], Douding Lu[3], Xiaoyong Shi[5], Chuansong Zhang[5]

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In 2005, CEOHAB (Ecology and Oceanography of Harmful Algal Blooms in China) carried out 5 cruises to further understand the mechanisms of large scale *Prorocentrum* blooms along east Chinese coast, near Changjiang river estuary and Zhejiang coast. The blooms re-occurred for several years at nearly the same time. Its occurrence was delayed in 2005, following a succession of bloom species, from *Skeletonema* to *Karenia* and then *Prorocentrum*. Preliminary clues were obtained to explain this phenomenon. First, physical oceanographic data showed that significant low temperatures of the seawater may have played an important role. The temperature of sea water at the end of March was about three degrees lower compared with 2004 and was not favourable for *Prorocentrum* to grow. Secondly, more nutrient input from excessive runoff of Changjiang river during winter might also play a role. And thirdly, different adaptive strategies caused diatoms to become dominant in the early spring, and the dinoflagellates later, so that *Prorocentrum* was delayed. Short-term climate change apparently could have a significant impact on the outbreak and succession of large-scale HABs along the east Chinese coast through alterations of environmental conditions.

PL.08
The physical oceanographic control of harmful algal blooms

Session: PL..08 - Plenary VIII – Ecology and Oceanography
Presentation time: 14:15 - 14:50

Robin Raine

The Martin Ryan Institute, National University of Ireland, GALWAY, Ireland

Many HABs impacting on aquaculture regions or other sites of amenity value arise because they are transported to the site. This occurs with local currents or else with water whose circulation is subjected to shifts in forcing variables such as the wind. A sound knowledge of coastal and near shelf physical oceanographic processes is therefore of immense importance if we are to predict HAB events with confidence. The effects of coastal currents, upwelling, and estuarine entrainment in transporting blooms have been acknowledged for some time. More recently, the influences of coastal and tidal fronts, wind forced water exchanges in bays, coastal jets and gyres have been studied in relation to HABs. Many coastal bays also have resident populations of HAB species. This situation arises not only due to a dormant sessile stage in the life cycle, but also because the balance between growth and physical dilution of the bay favours a bloom.
This paper reviews physical oceanographic processes within the context of HAB events using Irish coastal waters as an example. Data is also presented which

demonstrates that a good understanding of the physical processes which give rise to HABs can lead to robust, but simple, prediction models.

O.21-01
Wind patterns and HABs in upwelling systems

Session: O.21 - Ecology & Oceanography 2
Presentation time: 14:55 - 15:15

GC Pitcher[1], A Fawcett[2], S Bernard[2], AD Cembella[3], RM Kudela[4]

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[2]University of Cape Town, CAPE TOWN, South Africa
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[4]University of California Santa Cruz, SANTA CRUZ, United States of America

The southern Namaqua shelf of the Benguela upwelling system is frequently subjected to a variety of Harmful Algal Bloom (HAB) phenomena. Here winds dictate most physical processes important to the development of HABs. This presentation compares two periods of study (15 March – 16 April 2005 and 7 – 23 March 2006), following clearly different wind patterns. The 2005 study was dominated by dinoflagellates including species of *Dinophysis* and *Protoceratium reticulatum*, responsible for the production of DSP toxins and yessotoxins, respectively, while the 2006 study was dominated by several species of *Pseudo-nitzschia* known to produce domoic acid. The wind patterns leading to these very different bloom events are examined in terms of species selection and population dynamics. In particular the influence of the wind on the dynamics of the surface mixed layer, and on local upwelling processes determining across-shelf and alongshore flow, and frontal dynamics are investigated.

O.21-02
The multi-species nature of the 2005 Karenia bloom: implications for management and monitoring in Florida

Session: O.21 - Ecology & Oceanography 2
Presentation time: 15:15 - 15:35

CA Heil[1], E Truby[2], J Wolny[3], R Pigg[2], B Richardson[2], M Garrett[2], A Haywood[3], K Petrik[2], L Flewelling[2], S Cook[3], E Stone[2], KA Steidinger[3], J Landsberg[2]

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The severe 2005 *Karenia brevis* bloom was the first Gulf of Mexico-*Karenia* bloom in which multiple *Karenia* species were identified and monitored routinely. Five species were present over the course of the bloom: *K. brevis*, *K. mikimotoi*, *K. papilionacea*, *K. selliformis* and a recognized, but currently unnamed fifth species, *Karenia* sp. *Karenia brevis* was geographically widespread and numerically dominant over the entire bloom. Although *K. mikimotoi*, the second most common species, displayed similar geographical and temperature and salinity ranges as *K. brevis*, it was not present in significant concentrations until 4 months after bloom initiation. *Karenia selliformis*, also geographically widespread, was present only during the final bloom stages. Conversely, *K. papilionacea* was abundant only during the first two bloom months, and geographically restricted to offshore waters between St. Petersburg and Naples. *Karenia* sp. was further restricted, present only at higher salinities in the Ft. Myers to Naples area. These patterns of occurrence of the different *Karenia* species throughout the blooms suggest that *K. brevis* occupies the broadest ecological niche, with other species either spatially or temporally restricted. Management implications depend on impacts, toxins and toxicity of different strains. The first step is isolation of newly recorded species.

O.22-01
First evidence for the implication of nitric oxide in Ciguatera Fish Poisoning

Session: O.22 - Toxicology 3
Presentation time: 14.55 - 15:15

S Pauillac, F Vernel-Pauillac, S Kumar-Roine, M-P Sauviat, E Benoit, M Chinain, D Laurent

Institut Pasteur de Nouvelle-Calédonie, NOUMÉA, New Caledonia

The involvement of the nitric oxide (NO) pathway in ciguatera fish poisoning (CFP) has been investigated, in vitro and in vivo, in a ciguatoxin (CTX)/mouse model. The induction of inducible nitric oxide synthase (iNOS) synthesis at the mRNA level was kinetically measured using a real-time PCR protocol based on the LightCycler® technology. CTX-pulsed Neuro-2a cells (1 ng/mL) and peripheral blood mononuclear cells from CTX-injected mice (1ng/g), were demonstrated to express iNOS in a time-dependent manner. This strongly suggests that NO might be responsible for certain ciguatera symptoms (e.g. hypotension, allergenic effects and Chronic Fatigue Syndrome) which could not be solely explained by the activation of voltage-gated sodium channels. This hypothesis is supported by the observation that the most currently used drugs for the treatment of CFP are free radical scavengers.
In conclusion, the implication of NO in CFP paves the way for new therapies for both occidental and traditional medicines, together with new CTXs detection and clinical diagnostic tools.

O.22-02
Implications of saxitoxins for public health and natural resources in Florida

Session: O.22 - Toxicology 3
Presentation time: 15:15 – 15.35

JH Landsberg[1], JP Abbott[2], LJ Flewelling[1], PS Scott[1], JL Wolny[2]

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In early 2002, with the onset of puffer fish poisoning (PFP) originating from the Indian River Lagoon (IRL), saxitoxin was discovered in Florida and associated with *Pyrodinium bahamense* for the first time in the United States. Saxitoxins are usually associated with potentially fatal Paralytic Shellfish Poisoning (PSP), but prior to 2002, there was no public health risk from PSP in Florida. Since the detection of saxitoxins in puffer fish, the state initiated an intensive statewide monitoring program to determine concentrations and distribution of saxitoxins in biota. Because they are immune to saxitoxins, puffer fish can accumulate high toxin concentrations in the muscle, making them an extreme threat to consumers. Following FDA action levels (80 µg STX eq./100g meat) for acceptable limits of saxitoxins in seafood, the FWC banned puffer fish harvesting in the IRL. Apart from puffer fish that consistently exceed the action limit for saxitoxins, shellfish beds in the northern IRL were closed as a precautionary measure only on two brief occasions, just exceeding the acceptable limit by mouse bioassay. Preliminary evidence suggests that saxitoxins are present in the food chain but they remain an unknown risk to natural resources, despite documented mortality and disease events in the IRL.

SYMPOSIA, WEDNESDAY 6 SEPTEMBER

1. Ecosystem Disruptive Harmful Algal Blooms

ROOM: will be announced

Convener: Pat Tester

There is a new term in town – Ecosystem Disruptive Harmful Algal Blooms (EDABS) This session will explore the effects of eutrophication, relaxation of top down control and positive feedback loops that may help perpetuate EDABS. Ideas published in two recent papers will serve as the focus for this session that will include 2-3 speakers to introduce the topic and stimulate open, vigorous discussions about what EDABS are, what the feedback loops are and what consequences EDABS have to ecosystems viability.

Sunda et al. (2006) "Harmful algal blooms (HABs) have occurred with increasing frequency in recent years with eutrophication and other anthropogenic alterations of coastal ecosystems. Many of these blooms severely alter or degrade ecosystem function, and are referred to here as ecosystem disruptive algal blooms (EDABs). These blooms are often caused by toxic or unpalatable species that decrease grazing rates by planktonic and benthic herbivores, and thereby disrupt the transfer of nutrients and energy to higher trophic levels, and decrease nutrient recycling. Examples of EDAB species discussed in this paper include the pelagophytes *Aureococcus anophagefferens* Hargraves et Sieburth and *Aureoumbra lagunensis* DeYoe and Stockwell, the green tide algae *Nannochloris atomus* Butcher and *Nannochloropsis gaditana* Lubián, the haptophytes *Chrysochromulina polylepis* Manton et Parke *and Prymnesium parvum* Carter, and the cyanobacteria *Synechococcus elongates* Nägeli and *Nodularia spumigena* Mertens ex Bornet & Flahault. Many factors, such as nutrient availability and herbivore grazing have been proposed to separately influence EDAB dynamics, but interactions among these factors have rarely been considered. Here we discuss positive feedback interactions among nutrient availability, herbivore grazing, and nutrient regeneration, which have the potential to substantially influence the dynamics of EDAB events. The positive feedbacks result from a reduction of grazing rates on EDAB species caused by toxicity or unpalatability of these algae, which promotes the proliferation of the EDAB species. The decreased rates also lower grazer-mediated recycling of nutrients and thereby decrease nutrient availability. Since many EDAB species are well-adapted to nutrient-stressed environments and many exhibit increased toxin production and toxicity under nutrient limitation, positive feedbacks are established which can greatly increase the rate of bloom development and the adverse effects on the ecosystem. An understanding of how these feedbacks interact with other regulating factors, such as benthic/pelagic nutrient coupling, physical forcing, and life cycles of EDAB species provides a substantial future challenge."

Mitra and Flynn (2006) "The relationship between algae and their zooplanktonic predators typically involves consumption of nutrients by algae, grazing of the algae by zooplankton which in turn enhances predator biomass, controls algal growth and regenerates nutrients. Eutrophication raises nutrient levels, but does not simply increase normal predator–prey activity; rather, harmful algal bloom (HAB) events develop often with serious ecological and aesthetic implications. Generally, HAB species are outwardly poor competitors for nutrients, while their development of grazing deterrents during nutrient stress ostensibly occurs too late, after the nutrients have largely been consumed already by fast-growing non-HAB species. A new mechanism is presented to explain HAB dynamics under these circumstances. Using a multi-nutrient predator–prey model, it is demonstrated that these blooms can develop through the self-propagating failure of normal predator–prey activity, resulting in the transfer of nutrients into HAB growth at the expense of competing algal species. Rate limitation of this transfer provides a continual level of nutrient stress that results in HAB species exhibiting grazing deterrents protecting them from top-down control. This process is self-stabilizing as long as nutrient demand exceeds supply, maintaining the unpalatable status of HABs; such events are most likely under eutrophic conditions with skewed nutrient ratios."

Mitra, A. and K.J. Flynn. 2006. Promotion of harmful algal blooms by zooplankton predatory activity. Biology Letters.
http://www.journals.royalsoc.ac.uk/link.asp?id=pa6a316clj7hh

Sunda, W.R., E. Granéli and C.J. Gobler. 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. Journal of Phycology (on line August at
http://www.blackwellpublishing.com/journal.asp?ref=0022-3646&site=1

2. Human Health and HABs

Room: will be announced

Convener: Lorraine Backer and Heléne Annadotter

The first part of the symposium will address, using Florida red tide as an illustration, how marine harmful algal blooms (HABs) can affect coastal communities. In July, 2006, the National Oceanic and Atmospheric Administration, the Florida Fish and Wildlife Conservation Commission, Mote Marine Laboratory and the State of Florida Institute of Oceanongraphy hosted the workshop and public forum "State of the Research on Red Tide in the Gulf of Mexico." At the July workshop, speakers reviewed the progress in understanding this phenomenon in the contexts of *Karenia brevis* biophysiology, bloom dynamics and ecology, fisheries and food safety, oceanography, human health, economics, and community education and outreach. Red tides appear to be increasing in the frequency and intensity and are an ongoing threat to Florida's Gulf coast communities. In this workshop, there will be a brief presentation summarizing these community impacts that will set the stage for discussion about future directions for research on the effects of marine HABs on coastal communities.

The second part of the symposium will deal with the impact of cyanobacterial blooms on public health and the quality of drinking water. In the past decades, a number of reports of mass developments of cyanobacteria have appeared globally. A large number of documented incidents of death among animals, associated with cyanobacterial blooms, exist. Data on exposure of humans to cyanotoxins are limited. A range of symptoms among humans exposed to cyanobacteria in drinking water or in connection with swimming, have been documented. In most of these cases, the level of cyanotoxins were never measured. In a few cases, microcystins were measured but found to be around WHO´s safe level. In these cases, health problems such as fever, headache, skin rashes, abdominal pain, and muscle pain were reported. But which toxins and/or organisms are the cause of these different symptoms? The discussion will focus on the level of, and the role, of different cyanotoxins and cyanobacteria-associated organisms for the symptoms reported in connection with exposure of cyanobacteria in drinking water and during recreation.

3. Taxonomy – the species concept

Room: will be announced

Convener: Jacob Larsen

The species concept in different groups of protists will be introduced by invited speakers. During the session, we would like to stimulate a discussion of the species concept in the different groups of harmful algae: are cryptic species a common phenomenon? How are species defined in the future, by DNA bar codes or? Is species distribution restricted only by temperature?

Professor Tom Fenchel, University of Copenhagen, will give a general introduction to the species concept in protists while other speakers will talk specifically about different groups of harmful algae, e.g. *Karenia* and *Dinophysis*.

4. Toxicology of Toxin Analogues

ROOM: will be announced

Convener: Phillip Hess and John Ramsdell

The potential or known toxicity of toxin analogues is an issue of complexity. A number of organizations (incl. the EU) are active in the Codex Alimentarius work to clarify guidelines for national regulatory measures for toxin analogues.
The symposium will, after a few short introductory remarks, be an open discussion and exchange of new knowledge and viewpoints. We hope to obtain a state-of-the-art of current knowledge on toxicity of analogues, to discuss the TEF concept, as well as additivity and QSARs.
This symposium should particularly attract chemists, pharmacologists and toxicologists

5. HABs and Clay Flocculation: Some Species, Some Places, but not a Silver Bullet

ROOM: will be announced

Moderators:
Mario R. Sengco, Smithsonian Environmental Research Center
Kevin G. Sellner, Chesapeake Research Consortium, Inc.

For over a decade, natural clays have been used to control HABs in Japan, China, and South Korea, to minimize their impacts on aquaculture. Clays have also been used in Australia to treat *Microcystis* blooms in rivers and streams, and to remove phosphorus from the water column. In the U.S., clays have been shown to be effective against a number of marine and brackish-water species, although most studies have focused on *Karenia brevis* and its toxins in Florida waters. Other studies have also been conducted in Sweden, Hong Kong, and the Philippines.

Despite the growing number of investigations into the use of clays to control HABs, there has been little effort to consolidate our current understanding about the effectiveness of clays, the impacts of clay dispersal on water quality and the benthic environment, the cost of treatment, and the practical considerations of clay application. In this session, we have asked the speakers to summarize our current knowledge about the efficacy and impacts of clay flocculation, and to relate their experiences regarding the effectiveness, cost and implementation of this control strategy. We hope to provide a critical, scientific review of this method, as well as a balanced, practical discussion of its costs, benefits, and impacts.

Speakers:
Mario R. Sengco (Smithsonian Environmental Research Center, U.S.) – the use of clays against *Karenia brevis* and *Prymnesium parvum*

Kevin Sellner (Chesapeake Research Consortium, U.S.) – studies in Chesapeake Bay Monica Bricelj and Anne-Gaelle Haubois (National Research Council of Canada) – benthic impacts of clay applications

Chang-Kyu Lee (National Fisheries Research and Development Institute, South Korea) – the use of yellow clay against *Cochlodinium polykrikoides*

Zhiming Yu (Institute of Oceanology, Chinese Academy of Sciences, P.R. China) – recent clay applications in China

POSTER PROGRAMME

SESSION PO.01: GENETICS

SESSION PO.02: GENOMICS

SESSION PO.03: PUBLIC HEALTH

SESSION PO.04: FOOD CHAINS

SESSION PO.05: TOXIN ANALYSIS

SESSION PO.06: POPULATION DYNAMICS

SESSION PO.07: ECOLOGY AND OCEANOGRAPHY

SESSION PO.08: TOXICOLOGY

SESSION PO.09: TOXIN SYNTHESIS AND CHEMICAL STRUCTURE OF TOXINS

SESSION PO.10: ECOPHYSIOLOGY AND AUTECOLOGY

SESSION PO.11: ALLELOPATHY

SESSION PO.12: TAXONOMY AND PHYLOGENY

POSTER SESSION PO.13: REGIONAL EVENTS

SESSION PO.14: MITIGATION

SESSION PO.15: MONITORING

SESSION PO.16: LIFE CYCLES

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联合国教育、
科学及文化组织

UNESCO monographs on oceanographic methodology:

Real-time Coastal Observing Systems for Marine Ecosystem Dynamics and Harmful Algal Blooms: Theory, Instrumentation and Modelling

Edited by Marcel Babin, Collin Roesler and John Cullen

The proliferation of harmful phytoplankton in marine ecosystems can cause massive fish kills, contaminate seafood with toxins, impact local and regional economies and dramatically affect ecological balance. Real-time observations are essential for effective short-term operational forecasting, but observation and modelling systems are still being developed. This volume offers guidance for developing real-time and near real-time sensing systems for observing and predicting plankton dynamics, including harmful algal blooms, in coastal waters. It explains the underlying theory and discusses current directions in research and monitoring in looking at instrumentation and modelling.

Topics treated include: coastal ecosystems and dynamics of harmful algal blooms; theory and practical applications of in situ and remotely sensed optical detection of microalgal distributions and composition; theory and practical applications of in situ biological and chemical sensors for targeted species and toxin detection; integrated observing systems and platforms for detection; diagnostic and predictive modelling of ecosystems and harmful algal blooms, including data assimilation techniques; observational needs for the public and government; and future directions for research and operations.

This anthology should inform the work of researchers and environmental monitors as well as teachers and trainers concerned with understanding the causes, predicting the occurrences and mitigating the effects of harmful algal blooms in marine ecosystems.

Expected release October 2006. Will be announced at the ISSHA list server.

POSTER ABSTRACTS

PO.05-35
Liquid chromatography-tandem mass spectrometry techniques for the discovery of new marine algal toxin analogues and metabolites

Session: PO.05 - Toxin analysis

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[2]Institute for Marine Biosciences, NRC, HALIFAX, NOVA SCOTIA, Canada

In recent years the number of known marine algal toxins has increased tremendously, mainly by discovery of new analogues and metabolites within known toxin groups. This is due in particular to the use of LC-MS/MS. In this presentation, we will outline some new strategies based on various MS/MS scanning techniques that can be used for comprehensive detection and identification of new structural analogues of toxins. One example is the detection of assorted new analogues of the cyclic imine toxins, spirolides (SPX). Targeting key collision-induced product ions (m/z 150 or 164) in a precursor ion scanning mode and presenting the ion intensity data in a 2-D contour plot (m/z vs. retention time) allowed the detection of new toxins as individual 'spots' and also determines their masses. Product ion scans of those masses then allow detailed structure information to be generated. Thus, several new SPX analogues including fatty acid acyl ester metabolites have been identified. Examples will also be shown for the yessotoxin (YTX), okadaic acid (OA) and pectenotoxin (PTX) groups. The discovery of all these new toxin analogues raises some important questions such as their toxicological significance and whether we will be able to monitor for the toxins without standards.

PO.04-12
Statewide distribution of saxitoxins within Florida puffer fish species

Session: PO.04 – Food chains

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From January 2002 to April 2005, 28 cases of puffer fish poisoning (PFP) were reported, primarily from Florida. A harvesting ban was put into effect in April 2002. Previously unknown in Florida's marine waters, all incidents were due to saxitoxin present in the muscle of southern puffer fish (*Sphoeroides nephelus*) originating from the northern Indian River Lagoon (IRL) on Florida's Atlantic coast. Southern puffer fish in the IRL continue to pose a significant threat to public health with maximum levels in the muscle just over 20,106 µg STXeq./100 g tissue (n=384), (action level for shellfish is 80 µg STXeq./100g tissue). Lower levels, ranging from 10 to 3133 µg STXeq./100g tissue, have also been found in the muscle of southern puffer fish from Apalachicola (northwest), Jacksonville (northeast), Florida Keys (south), Charlotte Harbor (southwest), Tampa Bay (central west) and Tequesta (southeast). Checkered (*S. testudineus*) puffer fish from the Florida Keys, IRL and Tequesta (range= 17 to 137 µg STXeq./100g tissue) were generally much lower than southern puffers.

Bandtail pufferfish (*S. dorsalis*) muscle from Charlotte Harbor, Florida Keys, IRL, and Tequesta ranged from 1 to 1778 µg STXeq./100g tissue.

PO.08-18
Analysis of paralytic shellfish poisoning (PSP) toxins from mussels obtained from the Egyptian Coast

Session: PO.08 - Toxicology

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A sensitive HPLC method for determination of paralytic shellfish poisoning (PSP) based on ion-pair chromatographic separation of PSP toxins, post-column oxidation with periodic acid, and fluorescence detection has been used to determine toxin profiles of *Alexandrium minutum*. Total concentrations of PSP were 305 and 390 µg/100g mussels in *Mytilus* and *Donax*, respectively. The concentrations of decarbamoyl toxins in both species were much higher than the concentrations of carbomoyl toxins. In the case of the sum of STXeq, the concentration in *Mytilus* spp. 166 µg/100g mussels was higher (three fold) than that found in *Donax* spp. (64µg/100 g mussels). The most dominant toxin among the carbomoyl toxins in both species was CTX2. This is the first report in the literature of PSP toxin concentrations in mussels from the Mediterranean coast near Alexandria.

PO.08-26
Lack of effect of temperature on the depuration of domoic acid from the king scallop *Pecten maximus*

Session: PO.08 - Toxicology

CP Acosta[1], C Mariño[1], H Martín[2], J Blanco[1]
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Some previous observations, in laboratory and field studies, have suggested that temperature plays a foremost role in controlling the velocity of depuration of domoic acid from the scallop *Pecten maximus*. We maintained scallops at temperatures of 13 to 19 ºC during ca. 75 days and checked the effect of this factor on the domoic acid contents of four body compartments (digestive gland, adductor muscle, gonad and remaining tissues). No significant effect of temperature on the final domoic acid content was detected in any of the studied compartments nor in the total soft tissues. A slight increase in the toxins in the adductor muscle to the total pool of toxins was the only effect detected.

PO.15-09
DNA extraction method from harmful microalgae that is potentially applicable to an *in situ* quantitative real-time PCR detection

Session: PO.15 - Monitoring

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[2]MBARI, MOSS LANDING, United States of America

Various DNA extraction protocols are in use today for quantitative real-time PCR detection (QPCR) of HAB species. Co-purification of genomic DNA and compounds that inhibit PCR can confound analyses by producing false negative reactions. Here, we report on a new DNA extraction method for harmful microalgae that is potentially applicable for remote *in situ* applications using the Environmental Sample Processor (ESP). A QPCR assay for *Heterosigma.* (Raphidophyceae) was developed using species-specific primers and probe that target large-subunit rDNA. Filtration treatment with Supor (PALL, 0.22 μm) or Durapore (Millipore, 0.22μm) after addition of a CH3COOK solution post-lysis was highly effective at removing compounds that inhibit PCR. A GF/F filter was used for the solid phase extraction of DNA after that treatment. DNA was recovered with efficiencies similar to that obtained using the DNeasy Plant Mini Kit (Qiagen). A standard curve that has high linearity was constructed with genomic DNA extracted from cultured Heterosigma (1cell-10,000 cells) using the filter–based DNA extraction protocol. Results of this work suggest that this method is potentially applicable to in situ DNA extraction and amplification systems planed for future developments of the ESP.

PO.03-02
The economic consequences of red tide events on the Gulf Coast of Florida, USA

Session: PO.03 - Public health

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Red tide blooms (*Karenia brevis*) are naturally occurring events that frequent the Gulf of Mexico coast of Florida, USA. The exact causes of such events, including their duration and bloom intensity, are unknown. Studies directed toward understanding the economic impact red tides have on coastal communities have only recently been attempted. Two economic studies have been completed concerning red tide events along the Gulf of Mexico coast of Florida. One study attempted to assess the impact to local business activities along the southwest Florida coast. This study not only attempted to measure the changes in business activity within the region where the red tides occurred, but also attempted to assess the level of awareness that local residents had concerning red tide events. Another study attempted to measure the changes in business activity along the northern Gulf of Mexico coast of Florida. These studies represent the only empirical analyses addressing the economic consequences of red tide events in Florida, the state which has the greatest number of red tide events in the USA. Finally, an on-going study is currently measuring the economic impact of more recent red tide events within a larger region of the Gulf of Mexico coast of Florida.

PO.05-23
A *Microcystis aeruginosa* bloom and the occurrence of microcystins from a eutrophic freshwater lake in Comilla, Bangladesh

Session: PO.05 - Toxin analysis

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Bangladesh is a tropical country of large alluvial plains with 1.3 million freshwater ponds and lakes. It has a proper environment for growth of cyanobacteria and a bloom of *Microcystis aeruginosa* occurred recently in a lake in Comilla district. Methanol-water extracts of fresh bloom samples, filtered cells and lyophilized cells were analyzed by high performance liquid chromatography (HPLC) with UV detection and MS, and showed three types of microcystins viz., Microcystin-RR, Microcystin-YR and Microcystin-LR. Their presence was confirmed by HPLC-MS. In a fresh *M. aeruginosa* sample the amount of MC-LR was the highest (2.12 µg μl^{-1}) followed by MC-RR (1.4 µg μl^{-1}) and MC-YR (0.44 µg μl^{-1}). In case of lyophilized cells, the amount of MC-LR, MC-RR and MC-YR was 1048, 334 and 331 mg kg^{-1}. Acetic acid extracts of bloom samples were analyzed by the Thielert HPLC method but showed no paralytic shellfish poison. Histological studies of the liver of catfish, *Clarias batracus* from the contaminated lake showed damaged and deformed tissues, perhaps tumor-like structures. Further investigations are needed to characterize other types of microcystins from bloom-forming cyanobacteria and their effect on cultured fish in Bangladesh.

PO.11-01
Allelopathy in *Oscillatoria agardhii*: effect on monocultures of *Microcystis aeruginosa*

Session: PO.11 - Allelopathy

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Allelopathy refers to any direct or indirect, harmful or beneficial effect by one plant or microorganism on another through the production of chemical compounds that escape into the environment (Rice, 1984). This phenomenon is common in micro- and macroalgae, bacteria and virus, and it plays an important role in species interactions such as plankton succession, competition and bloom formation.
The allelopathic effect of cell-free spent media (SM) of non-toxic *O. agardhii* on monocultures of toxin-producing *M. aeruginosa* was investigated under controlled conditions using laboratory bioassays. Freeze-dried SM of *O. agardhii* was reconstituted to give 0.1, 0.05 and 0.02 g ml^{-1} spent medium in BG11 media. Aliquots of *M. aeruginosa* (10 % v/v) were added and incubated at 25 °C. Cultures were sampled at 3- and 6-week intervals to determine cell biomass and microcystin concentration. The results showed a concentration-dependent stimulatory effect of SM in cultures of *M. aeruginosa*. In addition, there was a significant increase in cell biomass, total- and intracellular microcystin per cell weight.

Reference

Rice, E. L. (1984) Allelopathy. Second Edition. Academic Press, Orlando. 189 – 205.

PO.15-26
HAB-MAPS of toxic marine microalgae in the 'Cono Sur' of South America

Session: PO.15 - Monitoring

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Information on the distribution of harmful microalgal species and their changes over time is essential to evaluate if harmful events are expanding/contracting in a given region, and to interpret dispersal routes. This information is also useful for risk assessment and planning of the exploitation of marine resources. The ISSHA project HAB-MAP was created to establish a global referenced map of the known distribution of toxic marine microalgae species. Here we present HAB-MAP activities for the coastal waters of the Cono Sur of South America (Brazil, Uruguay, Argentina, Chile, Perú and Ecuador) on the basis of a literature review that comprised 150 published papers and reports. The database was developed with Excel-files for each species; different worksheets provided data-sets extracted from each bibliographic reference. Factors considered included geo-referenced location (to allow the generation of distribution maps), date, cell abundance, toxin content, harmful effects and relevant environmental information. A total of 40 toxic species - 9 diatoms, 23 dinoflagellates, 3 haptophytes and 5 raphidophytes- were recorded. The total number of toxic species could be greater than the apparent one because of dubious taxonomic identification of some taxa, and low frequency of sampling in large areas of the Cono Sur.

PO.13-44
Phytoplankton distribution, diversity and nutrient variations at the west coast of Sweden, with special reference to harmful algae

Session: PO.13 - Regional events

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Species composition, abundance and distribution of phytoplankton as well as some major physico-chemical features and chlorophyll a concentration were investigated along the Swedish Skagerrak coast. This study is part of a long-term program for monitoring of harmful algae along the Swedish coast. Owing to the eutrophic nature of the region, phytoplankton is more productive than diversified. Diatoms, dinoflagellates and some other flagellates dominate phytoplankton populations. Highest abundance was in Koljöfjord reaching 3.32 x 10^6 cells^{-1} in May 2005, with diatoms constituting 69.7%. Lowest abundance was in Kosterfjord in March 2006, when cell densities had dropped to 11.7 x 10^3 cells l^{-1}. Diatoms were exceptionally scarce at this station in May 2005 (0.150 x 10^3 cells l^{-1}) accompanied by a dominance of the harmful dinoflagellate *Dinophysis norvegica* and some cryptophytes. Dinoflagellates reached their highest abundance of 1.18 x 10^5 cells l^{-1} at Koljöfjord in August 2005. Chlorophyll a reached a minimum of 1.1 µg/l in April 2005 (Danafjord) and a maximum of 8.1 µg/l in September (Kosterfjord). A number

of harmful algal taxa incidently appear in this region, these include species belonging to *Alexandrium*, *Dinophysis*, *Pseudo-nitzschia* and *Chattonella*.

PO.13-27
The effect of *Noctiluca scintillans* on harmful algal species of south eastern Australia

Session: PO.13 - Regional events

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The red tide-forming dinoflagellate *Noctiluca scintillans* has bloomed off eastern Australia since the 1970s and was first recorded in Tasmanian waters in 1994. The distribution of *N. scintillans* blooms in Tasmanian waters over the time period of 2001 - 2005 was investigated with monthly monitoring in the D'Entrecasteaux Channel and the Huon Estuary from September 2004 to January 2005, determining population fluctuations in the area. The bloom frequency in Tasmanian waters showed an increase between 2001 and 2004, and there was an increasing southwards distribution in the D'Entrecasteaux Channel and the Huon Estuary with the peak abundance in December. *Noctiluca scintillans* feeds on a variety of prey items. In this study we investigated the feeding preferences of *N. scintillans* when fed ecologically important harmful algal species (HABs) of south eastern Australia. The feeding experiments showed that *N. scintillans* has the potential to graze both toxic and non-toxic species, and the ability to ingest and thrive on HAB species such as *Gymnodinium catenatum* and *Chattonella antiqua* (specific growth rates approx. 0.3 divisions day^{-1}) suggests that *N. scintillans* can have a major impact on the microalgal populations of south eastern Australia.

PO.13-06
Benthic species of the genus *Prorocentrum* Ehrenberg in the eastern Mediterranean Sea (North Aegean Sea, Greece)

Session: PO.13 - Regional events

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Benthic *Prorocentrum* species constitute a subject of interest since most of them are associated with toxin production. In order to provide additional information on the occurrence of these species, a survey on 50 sites along North Aegean continental and island coasts was conducted in August 2003-December 2005. At least five different benthic *Prorocentrum* species (*P. lima*, *P. emarginatum*, *P. rhathymum*, *P. borbonicum*, *Prorocentrum* sp1.) were recorded. *Prorocentrum borbonicum* represents a new record for the Mediterranean algal flora; *Prorocentrum* sp1. does not fit any of the presently described benthic *Prorocentrum* species, while *P. lima*, *P. emarginatum* and *P. rhathymum* have been recently recorded in the Eastern Mediterranean Sea. Species identification was based on light and electron microscopy of preserved field material and clonal cultures. *P. lima* contributed remarkably to the benthic dinoflagellate community, which comprised also *Ostreopsis ovata*, *O*. cf. *siamensis*, *C. monotis* and *Amphidinium* spp.; *P. lima* was

detected throughout the year achieving abundances up to $1.33x10^5$ cells g^{-1} fwm epiphytically on the marine phanerogam *Cymodocea nodosa* in November, while the presence of the other *Prorocentrum* species was more scarce and restricted to the warm period.

PO.13-17
Occurrence of species from the genus *Pseudo-nitzschia* in the southwestern Atlantic and Southern Ocean

Session: PO.13 - Regional events

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The distribution of *Pseudo-nitzschia* species and its relationship with main environmental factors were studied in surface waters of the Argentine and Weddell Seas (38-55°S and 61-77°S, respectively). Both qualitative and quantitative samples, collected during summer and fall 2003, summer 2004 and spring 2005 were examined using light and scanning electron microscopy. Several *Pseudo-nitzschia* species were found in the Argentine Sea, including *P. americana*, *P. australis*, *P. calliantha*, *P. fraudulenta*, *P. heimii*, *P. lineola*, *P. pungens*, *P. subcurvata*, *P. turgidula* and *P. turgiduloides*. *Pseudo-nitzschia pungens* and *P. australis* were the most common species recorded, especially in waters with elevated temperatures and salinities (around 15 ºC, 33.8psu) and low nutrient concentrations. The rest of the species showed a more restricted distribution. *Pseudo-nitzschia calliantha* and the epiphytic *P. americana*, both reported for the first time in Argentinean waters, were found only during spring. A lower number of species was found in the cold (0.6 to -1.6 ºC), nutrient-rich waters of the Weddell Sea. Notably, *P. lineola*, *P. prolongatoides*, *P. subcurvata* and *P. turgiduloides* showed a widespread distribution, while *P. heimii* and *P. turgidula* were confined tp northward of 62ºS. Morphometric data, abundances and ecological preferences are given and compared with previous studies.

PO.02-07
New insights into the higher order organization of the dinoflagellate chromosomes: evidence of eukaryotic differentiations

Session: PO.02 - Genomics

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Dinoflagellates are eukaryotic protists with a unique genome and chromosome organization. They lack histones and nucleosomes, being therefore considered the only living knockouts of these proteins. They have distinct interphasic chromosomes stabilized by metal cations and DNA-RNA hybrids. Their DNA has a high G+C content, highly methylated and rare bases and their genomes lack the canonical TATA box, showing specific regulatory sequences in their promoters, as well as linear arrangement of genes and gene

families.
Without histones and with a low proportion of histone-like proteins, dinoflagellates need a different way of packing their huge amounts of DNA into functional chromatin. Despite the interest in their genome, studies on the molecular organization of their chromosomes are scarce.
FISH with telomeric and rDNA gene sequences were applied to three dinoflagellates species. Our results revealed the clustering of rDNA genes in NORs and the presence of distal telomeres, which confirms the linear organization of their chromosomes. They also showed specific features such as the presence of non-randomly distributed structural RNA in the chromosome body. These results provide the first evidence of eukaryotic differentiation of these unique chromosomes, reinforcing the dinoflagellates' position within the eukaryotic lineage.

PO.05-05
Lipophilic toxins in French shellfish: first report of pectenotoxin-2, spirolide-C and their isomers by liquid chromatography/mass spectrometry

Session: PO.05 - Toxin analysis

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IFREMER, NANTES CEDEX 3, France

During the French Phytoplankton and Phycotoxins monitoring network (REPHY), shellfish samples were harvested from different locations where harmful algae bloom occurred. For all shellfish samples found positive for diarrheic shellfish poisoning (DSP) toxins by mouse bioassay, liquid chromatography (LC) coupled with mass spectrometry (MS) was used to search for the following lipophilic toxins: okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs), azaspiracids (AZAs), yessotoxins (YTXs), spirolides (SPXs) and gymnodimine (GYM). In order to investigate the presence of okadaic acid esters, alkaline hydrolysis was performed on all samples, and LC/MS analyses were carried out on the samples before and after hydrolysis. The results revealed different lipophilic toxin profiles depending on shellfish sampling locations. The primary finding was that all samples contained okadaic acid and its acyl-ester derivatives (DTX-3). In addition, other lipophilic toxins were found in the shellfish samples: DTX-2, its acyl ester derivatives (DTX-3) and spirolides (SPXs) on the Atlantic coast (Southern Brittany, Arcachon), pectenotoxins (PTX-2, PTX-2-Seco-acid) on the Mediterranean coast (Thau Lagoon, Corsica Island). This paper is the first occurrence of pectenotoxin-2, spirolide-C-deMe and their isomers in France.

PO.13-24
Harmful algal blooms and eutrophication: nutrient sources, composition and consequences in the Arabian Gulf bordering Abu Dhabi Emirate

Session: PO.13 - Regional events

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Environment Agency, ABU DHABI, United Arab Emirates

The Arabian Gulf has witnessed an increase in algal bloom incidents over the last decade, presumably due to pollution from land reclamation and urbanization. In response to growing concerns over the impact of harmful algal blooms on marine resources, ecosystems and human health, a survey was initiated to study the harmful algae of the waters of Abu Dhabi Emirate bordering the Arabian Gulf. A harmful algal bloom was recorded in one of the sampling areas (Mussafah). It had a maximum cell concentration of 18 x 10^7 cells/L and showed seasonal variation in species dominance: dinoflagellates during winter and cyanobacteria during summer. The winter bloom was represented by *Prorocentrum* spp and the water was brown in colour whereas in summer the bloom was generated by *Oscillatoria* sp., and the water had a green colour. The hydrographic parameters showed abnormal conditions. Nutrient values were many folds higher than the normal seawater and confirmed the eutrophic nature. The blooms were associated with widespread harmful impacts including hypoxic events (0.20mg/L), finfish kills (*Nematalosa nasus*) and subsequent loss of benthic organisms. The sources, composition and consequences of nutrients and bloom formation and species dominance in Abu Dhabi waters will also be discussed.

PO.16-01
Local importance of resting cysts for a dinoflagellate bloom initiation

Session: PO.16 – Life cycles

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Resting cysts are the product of sexual reproduction of dinoflagellates and comprise the normal dormant phase of these organisms. Cysts may survive in the sediment for months or even years, and during that time their dispersion and concentration is influenced by many factors. In the case of localized blooms in shallow, restricted embayment or harbours, the coupling between cysts in the sediment and blooms in the water is direct. Recurrent blooms of the toxic dinoflagellate *Alexandrium minutum* in the 'Arenys de Mar' Harbour (NW Mediterranean) provided a perfect opportunity to study resting cyst dynamics and to evaluate the number of cysts that contribute to the bloom initiation. We quantified the resting cysts of *A. minutum* and other dinoflagellates in sediment samples collected during several cruises and evaluated their spatial and temporal distribution patterns during pre- and post-bloom conditions. We also studied germination rates of *A. minutum* cysts at different locations in the harbour. Standard sedimentary variables (grain size, coarse and fine fraction, organic and inorganic C, organic N), as well as physical and meteorological parameters were considered for interpretation of observed variability in cyst concentrations.

PO.03-09
A water-asssociated dermatitis in Swedish lakes

Session: PO.03 - Public health

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In 1999, an outbreak of water-associated dermatitis in the Swedish Lake Värsjön was found to be caused by schistosome cercariae using the snail *Radix*

(=*Lymnea*) *peregra* as the intermediate host. In connection with the outbreak, the cyanobacterium *Anabaena lemmermannii*, and the flagellate *Gonyostomum semen* dominated the algal community.
In a survey of 25 recreation lakes in Scania, Southern Sweden, schistosome cercariae were found in four of these lakes. In all four lakes, mass developments of cyanobacteria alone or combined with *Gonyostomum semen* occurred regularly during the vegetative season.
Increased endotoxin levels in lake water may be associated with developments of cyanobacteria and/or *Gonyostomum semen*. A study of endotoxins in *Gonyostomum*-dominated lakes revealed high concentrations; 220-1880 EU ml^{-1}. Endotoxins in the blood stream of the bird-host may stimulate the production of the cytokine tumour necrosis factor (TNF). TNF may, in turn, stimulate egg-laying of the schistosome parasite as well as the excretion of the parasite eggs from the host. We suggest that increased endotoxin concentrations in the water are triggering the occurrence of schistosome cercariae in lakes.

PO.13-47
Paralytic shellfish poisoning and food web contamination: a California coastal example

Session: PO.13 - Regional events

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The central California coast is a highly productive, biodiverse region frequently affected by harmful algal blooms (HABs). In California, shellfish are regularly monitored for the presence of Paralytic Shellfish Poisoning (PSP) and domoic acid toxins to protect human consumers, and there are data here, as in other coastal areas around the world, showing that additional marine organisms are contaminated by these same toxins. In the present study our primary goal is to understand the extent to which PSP toxins are contaminating local commercial fisheries, if at all. Here we investigate several commercially important species, including planktivorous pelagic fish, a variety of flatfish, shellfish and rock crab. Three years of data on the particulate saxitoxin concentrations and the cell abundance of *Alexandrium catenella*, the alga responsible for PSP in this region, will be compared to the presence, or lack thereof, of toxins in these organisms. The presence of saxitoxin in several of these organisms suggests that PSP toxins are more prevalent in California food webs than previously thought. In addition to PSP, we will report on the potential for these same organisms to be simultaneously contaminated with multiple phycotoxins, specifically domoic acid and/or okadaic acid.

PO.05-40
Content and profile of lipophilic toxins in plankton samples during two *Dinophysis acuta* outbreaks, in Galician Rías (NW Spain)

Session: PO.05 - Toxin analysis

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In order to assess the variability in both content and profile of lipophilic toxins per *Dinophysis acuta* cell, two toxic outbreaks were studied, in which this toxic species was the dominant one. In the first bloom (2001) water samples were collected by net tow (from 0 to 15 meters) in three nearby stations, on 3 October. In the second one, (autumn 2005), plankton samples were collected weekly at three depth intervals (0-5, 5-10 and 10-15 meters) using a hose. The toxin content of the plankton samples was analysed, in the first case by HPLC-FD, and in the second by LC-MS, and in both cases phytoplankton was quantified in a inverted microscope. The toxin content per *D. acuta* cell was independent of cell concentration and ranged from 0.20 to 10.8 pg·cell^{-1}, without significant differences between the two episodes. In the samples from 2001, OA, its conjugated forms and pectenotoxins were detected, with a slight predominance of OA-conjugated forms. During 2005, OA, DTX2 and the conjugated forms of both toxins were found in very variable proportions, even between different depths during the same day, but with a slight predominance of free OA.

PO.13-71
Foam events due to a *Phaeocystis* bloom along the Catalan coast (NW Mediterranean)

Session: PO.13 - Regional events

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Marine haptophytes of the genus *Phaeocystis* may produce nearly monospecific blooms in many nutrient-rich areas. Dense blooms of *Phaeocystis* colonies in coastal zones are frequently associated with extensive masses of foam, causing problems for local fishing activities and tourist industries. In March 2006, a *Phaeocystis* bloom occurred along the Catalan coast (NE Spain). The bloom was associated with foam formation and extended several kilometres in coastal waters. Based on the observed morphology of the colonies, the responsible species was identified as *P. globosa*. The bloom was not monospecific as diatoms were also abundant (mostly members of the genera *Rhizosolenia*, *Chaetoceros*, *Pseudo-nitzschia* and *Bacteriastrum*). *Phaeocystis* has previously been recorded along the Catalan coast, but not at such high densities. On this occasion, the abundance of cells was high enough to produce visible amounts of foam that could, for the first time, be recorded by ARGUS video cameras installed in the area.

PO.06-11
Gymnodinium catenatum preference for and growth on nitrate, ammonium and urea

Session: PO.06 - Population dynamics

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The toxic dinoflagellate *Gymnodinium catenatum* has formed recurrent blooms in the Huon Estuary, south east Tasmania since the mid 1980s. Phytoplankton growth in the Huon Estuary is primarily limited by nitrogen (N). To better understand the bloom dynamics of *G. catenatum*, preference for and growth on: nitrate ($NO3^-$), ammonium ($NH4^+$) and urea was investigated in a series of laboratory experiments. Results demonstrated that *G. catenatum* grows equally well on: nitrate, ammonium and urea, but takes them up in this order: $NH4^+$ > $NO3^-$ > urea. In addition, *G. catenatum* is capable of initial specific N uptake rates ~20 times its specific growth rate i.e. 'surge uptake'. The ability of *G. catenatum* to grow equally well using all three N sources coupled with 'surge uptake' capability are likely to play an important role in its dominance in the Huon Estuary and other ecosystems.

PO.13-33
Prolonged toxicity of *Scrobicularia plana* after PSP events and its relation to *Gymnodinium catenatum* cyst consumption and toxin depuration

Session: PO.13 - Regional events

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In contrast to other species of bivalves such as blue mussels (*Mytilus galloprovincialis*) and common cockles (*Cerastoderma edule*), the clam *Scrobicularia plana* has been observed to retain PSP toxicity for long periods (more than 1 year) after a bloom event. Because this species is a deposit feeder, consumption of *Gymnodinium catenatum* cysts from the sediments was suggested previously as one possible explanation for this fact. In autumn 2005, a bloom of *G. catenatum* was detected along the NW coast of Portugal. The gut contents of *S. plana* from affected areas were examined for dinoflagellate cysts during and after the bloom. The PSP content in bivalves was followed by HPLC. The presence of cysts was maximal during the bloom and then deminished, even though PSP toxicity never decreased to values below the regulatory safe limit. Depuration experiments with *S. plana*, blue mussels and common cockles revealed that elimination of toxins in *S. plana* was almost non-observable within 1 week, while in mussels or cockles reduction to half the toxin level took place. These results suggest that cysts are not responsible for the observed prolonged toxicity, but instead a slow depuration rate.

PO.10-35
Nitrogen dynamics of *Pseudo-nitzschia cuspidata* from the U.S. Pacific Northwest

Session: PO.10 - Ecophysiology & autecology

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The growth capabilities of the toxigenic diatom species *Pseudo-nitzschia cuspidata*, isolated from the Juan de Fuca Eddy region during Fall surveys of the ECOHAB-PNW project in 2004 and 2005, were examined in non-axenic, semi-continuous, batch cultures enriched with 40 µM nitrate, 40 µM ammonium or 20 µM of urea as the sole nitrogen source. Experiments, conducted at both high (120 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and low (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) photosynthetic photon flux densities (PPFDs), demonstrate that *P. cuspidata* grew significantly faster at the higher PPFD, but shows no preference for one nitrogen source over the other at either PPFDs. Exponential growth rates (determined using cell abundance over time) and particulate domoic acid content (using ELISA) did not significantly differ as a function of the nitrogen growth substrate. In contrast to most previous studies using other cultured *Pseudo-nitzschia* species, the particulate DA per cell averaged 60% greater during exponential growth compared to stationary growth of *P. cuspidata*, regardless of nitrogen source or PPFD. These results demonstrate the capability of this diatom to grow equally well on both oxidized and reduced forms of nitrogen, although our field observations have generally found greatest cell abundances in the nitrate-rich upwelled waters of the Juan de Fuca Eddy.

PO.13-65
Cyanobacterial toxins in the lakes located in the Riga City and its surroundings

Session: PO.13 - Regional events

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The coastal zone of the lakes located in the Riga City and its surroundings are densely inhabited and widely used for recreation, fishing activities and as drinking water source for Riga City. Due to strong anthropogenic impact, intensive blooms of potentially toxic algae in the lakes are observed every summer, however no regular analysis of algal toxins has been carried out. In this study we attempted to quantify the toxin concentrations in different stages of cyanobacterial blooms in 2002-2005. Samples for cyanobacterial toxin analysis were collected from algae surface accumulations. Presence of toxins was detected by protein phosphatase inhibition assay (PPIA), enzyme linked immunosorbent assay (ELISA) and HPLC. The most frequently occurring species in all samples were *Microcystis* spp., *Anabaena* spp. and *Aphanizomenon flos-aquae*, composing 50 -95% of the total phytoplankton biomass. The highest concentrations of microcystins were detected in the lakes Mazais Baltezers and Lielais Baltezers, ranging from 300- 1600 ng/mg dw during the maximum of the cyanobacterial bloom. These results suggest that more public attention should be paid to the problem of HAB, to avoid possible intoxication cases.

PO.08-04
PSP toxin profiles during different growth phases in *Gymnodinium catenatum* strains isolated from the Gulf of California, Mexico

Session: PO.08 - Toxicology

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In vitro experiments were performed with *Gymnodinium catenatum* Graham strains isolated from the Gulf of California (Bahía de Mazatlán, Bahía de La Paz, Bahía Concepción) to determine the variability in toxicity and toxin profile during different growth phases. Growth rates varied between 0.70 and 0.82 day^{-1}. Highest cell yields were reached at 16 and 19 days with densities between 1090 and 3393 cells mL^{-1}. Mazatlán and La Paz strains were more toxic (101 pg STX eq cell^{-1}), compared with strains from Concepción (13 pg STX eq cell^{-1}). Toxin content did not change with culture age (0.2 - 0.6 pmol PSP cell^{-1}). All strains contained NEOSTX, dcSTX, dcGTX2-3, B1-2, and C1-2 toxins. Concepción strains had the highest content of C1, whereas Mazatlán and La Paz strains had a higher percentage of NEOSTX (23 to 52%). Mazatlán and La Paz strains showed differences in toxin composition with culture age. Cultures with higher percentage of long chains had more NEOSTX, while those with a higher proportion of short chains had a lower content of NEOSTX. Gulf of California strains are characterized by a high proportion of NEOSTX, and seem to have evolved particular physiological responses to their environment that are reflected in the toxin profile.

PO.10-16
Metal concentration in freshwater sediments seasonally subjected to toxin-producing cyanobacterial blooms

Session: PO.10 - Ecophysiology & autecology

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Cyanobacteria represent a sink for metals in aquatic environment, as they are effective metal sorbents, therefore affecting metal speciation and bioavailability. In the ambit of a project, aiming at investigating causes and consequences of cyanobacterial blooms in freshwater, trace metal (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) concentrations in sediments were monitored monthly, throughout one year, at two sites of Tâmega River (Northeast Portugal): (1) the Marco reservoir, which has been seasonally dominated by toxin-producing strains of *Microcystis aeruginosa*, from June to September; and (2) the city of Amarante (ca. 20 km upstream) where no such blooms have been recorded. The results show the baselines of trace metal concentration to be similar for both sites, in spite of site (1) being a lentic system whereas site (2) is a lotic one. However, extreme draught characterized the sampling year; its influence on the results remains to be ascertained. Maxima of metal concentrations were registered at both sites during the bloom event, which, unlike previous years, was recorded at site (1) only during October and November (no phytoplankton bloom was recorded

at site (2)). The implications of these results to the metal dynamics in Tâmega River will be discussed.

PO. 10-39 Effect of carbonate addition on domoic acid production by *Pseudo-nitzschia multiseries* in batch culture

Session: PO.10 - Ecophysiology & autecology

Bates SS, Léger C

Several factors control the production of the neurotoxin domoic acid (DA) by the diatom *Pseudo-nitzschia multiseries*, including silicate and phosphate limitation, irradiance level, presence of bacteria, concentration of copper and iron, and pH. Most DA is produced post-exponential phase. At that time, the concentration of CO_2 has decreased due to photosynthetic uptake, pH is high, and the carbonate system has shifted toward higher proportions of bicarbonate and carbonate. It is therefore possible that total inorganic carbon (TIC) may limit DA biosynthesis. To test this, medium f/2 (containing 1.9 mM TIC) was amended with sodium bicarbonate to give 2.8 and 3.7 mM inorganic carbon. Stationary-phase cell numbers were similar at the three initial carbon concentrations. TIC decreased during the exponential phase and generally increased (unexpectedly) during stationary phase. pH changed in an inverse fashion, reaching a maximum of 8.95, for the highest TIC concentration, at the end of exponential growth, and then declined during stationary phase. Highest DA was associated with highest pH.
Cellular DA concentration increased linearly with increasing initial TIC concentration (r^2 = 0.983), suggesting carbon limitation of DA biosynthesis. Agitation on a rotary shaker table increased growth rate and also DA production. These conditions may be used to boost DA production in batch cultures.

PO.06-01 Antibiotic synthesis by the bacterium *Silicibacter* sp. TM1040 is involved in the formation of obligate symbiotic interactions with dinoflagellates

Session: PO.06 - Population dynamics

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Members of the *Roseobacter* clade of the a-Proteobacteria are among the most abundant and ecologically relevant marine bacterial groups. One of the most salient features of these bacteria, such as *Silicibacter* sp. TM1040, is their ability to metabolize dinoflagellate-derived dimethylsulfoniopropionate (DMSP), a major source of organic sulphur in the ocean. *Silicibacter* sp. TM1040 forms an obligate symbiotic relationship with DMSP-producing dinoflagellates and phytoplankton suggesting that the bacteria are highly adapted to engage in both positive and negative interactions with other marine microorganisms. *Silicibacter* sp. TM1040 actively metabolizes DMSP, and is chemotactically attracted to dinoflagellate homogenates, DMSP, amino acids, and other chemicals released by the dinoflagellate. Little is known about the cellular factors and molecular mechanisms required for roseobacters to establish and maintain their symbiosis with dinoflagellates. Recently, we discovered that *Silicibacter* sp. TM1040 produces an antibacterial compound, tropodithietic acid, which inhibits many other marine bacteria. A genetic analysis has revealed the biosynthetic pathway, as well as the regulatory circuit controlling tropodithietic acid expression. The implications of these data and the effect of the antibiotic on the interaction of roseobacters with their dinoflagellate hosts, the structure and composition of bacterial communities, and the sulfur cycle are discussed.

PO.13-28
Phytoplankton tidal population in Sfax coasts (South Tunisia)

Session: PO.13 - Regional events

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Diversity variation of phytoplankton in relation to daily tidal movements was studied during four seasons at the Sfax coast, Tunisia. Diatoms that adhere to the bottom move with in the water column and can reach the surface. The most abundant groups of the phytoplankton were dinoflagellates and some cyanophytes. Diatom abundance has a lunar as well as a seasonal variability, with the highest abundance corresponding to the coldest days of the lunar cycle. Dinoflagellate species occurred generally in summer and proliferated specially on the last days of the lunar cycle, showing a preference for decreased turbulence.

PO.10-46
Influence of salinity on the dimensions of the dinoflagellate *Prorocentrum minimum* under controlled conditions

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Prorocentrum minimum is a planktonic dinoflagellate, which is considered potentially toxic to humans. Associations between *P. minimum* blooms and toxicity are rare, but harmful effects were on various occasions observed in areas of *P. minimum* blooms. *Prorocentrum minimum* is therefore considered a HAB (Harmful Algal Bloom) species and a species to be monitored.
Prorocentrum minimum cell size and shape are highly variable, and different factors, including salinity, temperature and light intensity, have been suggested as putative causes for this variability. Various authors have suggested that different forms could be treated as morphotypes within the species, and relationships between *P. minimum* morphology, geographical distribution and salinity have been hypothesized. To study the possible relation between cell dimension and salinity, *P. minimum* strains from different geographical areas were maintained in culture under controlled conditions. Growth curves were monitored at three salinities (8, 16 and 32 PSU) and cell dimensions analyzed. Cell dimensions varied in the different phases of growth, and the average

cell size depended on the strain used and not on the salinity of the growth medium.

PO.15-06
PCR-based monitoring of toxic dinoflagellates in a Mediterranean shellfish farm

Session: PO.15 - Monitoring

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Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP) are syndromes caused by consumption of shellfish contaminated with toxins produced by several species of marine dinoflagellates. The standard monitoring of shellfish farms for the presence of harmful algae and related toxins usually requires microscopic examination of phytoplankton, bioassays and toxin determination by HPLC. Molecular biology techniques may be helpful in the detection of target microalgae in contaminated mussels.
A qualitative PCR assay for the rapid detection of the genera *Alexandrium* and *Dinophysis* in seawater samples and mussel tissues was developed. This method was tested in a monitoring program in a shellfish farm along the NW Adriatic Sea and Ionian coast of Italy during 13 months. In seawater samples, the PCR method has generally shown a greater sensitivity compared to microscope analysis. Instead, the detection of genera *Alexandrium* and *Dinophysis* in mussel tissues poorly reflected the presence of these dinoflagellates in the corresponding seawater samples. However, no DSP toxicity was found in mussels during the entire monitoring program. Even if no correlation was found between the presence of algal DNA and toxin accumulation in mussels, the detection of harmful algae DNA could furnish an early warning for potential mussel toxicity

PO.02-06
Molecular physiology of the toxigenic haptophyte *Prymnesium parvum*

Session: PO.02 - Genomics

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Prymnesium parvum is a toxic haptophyte, which forms harmful blooms causing mass fish mortalities in brackish and marine waters. A functional genomic approach, within the EU project ESTTAL (expressed sequence tags of toxic algae), is being used to investigate the basis of toxicity and growth regulation in this species. About 9,000 clones randomly selected from a normalized cDNA library were sequenced and approximately 15,000 ESTs have been generated. The resulting ~6230 contigs were analysed in silico and classified into functional categories. The ESTs served as the basis for oligonucleotide design to produce a microarray for gene expression analysis. *Prymnesium* toxins, known as prymnesins, are linear polyether compounds suggesting that are produced or at

least partially modified via polyketide biosynthetic pathways. Therefore, our research focussed upon polyketide synthase (PKS) genes. Six potential candidate PKS sequences were identified among the ESTs based on similarity searches. We also performed physiological experiments to monitor toxicity, allelochemical potency and growth of *Prymnesium parvum*. RNA was extracted from different treatments and physiological stages of the cultures to monitor gene expression with microarrays. Special focuses of our investigations are the genes related to growth regulation and toxin synthesis.

PO.13-26
Potentially toxic microalgae from coastal lagoons along the middle Tyrrhenian Sea (Mediterranean Sea)

Session: PO.13 - Regional events

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A complex system of six coastal lagoons (Fogliano, Monaci, Caprolace, Sabaudia, Fondi and Lago Lungo) located along the Southern Latium coast was monitored for algal surveillance and environmental assessment from January 2003. These lagoons showed high biomass potential (up to 70 µg l^{-1} chl a) and were markedly influenced by marine and freshwater influxes and host fisheries and shellfish farms. Phytoplankton composition and spatio-temporal distribution was assessed in monthly samples by light and electron microscopy, revealing similar trends among the different site populations. Seven potentially toxic dinoflagellates were detected, with maximal abundance ($8x10^6$ cells l^{-1}) of Prorocentrum minimum at Fondi during spring. Mass occurrences of *Prymnesium* cf. *parvum* ($92x10^6$ cells l^{-1}) and *Pseudo-nitzschia* species ($24x10^6$ cells l^{-1}) were recorded at Fondi and Lago Lungo, respectively. Despite the variety and density of potentially toxic species, fish mortality was only registered in October 2004 during a *Prymnesium* cf. *parvum* bloom ($92x10^6$ cells l^{-1}). In addition, monitoring of phytoplankton from the Orbetello Lagoon, at South Tuscany, over a one-year period showed unusual and persistent winter blooms of *Dinophysis sacculus* and *Prorocentrum micans* (up to 26 and $30x10^6$ cells l^{-1} respectively). Toxicity tests on farmed oysters revealed the presence of DTX-3 by LC-MS/MS.

PO.05-03
The use of biopsies to quantify domoic acid concentration in the king scallop *Pecten maximus*

Session: PO.05 – Toxin analysis

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Several biopsy methods were checked to obtain samples of several organs of the king scallop *Pecten maximus*. Aspiration methods or open concentric needles were found to be inefficient for samples of digestive gland, gonad and adductor muscle. Cutting devices gave good, consistent samples of the three organs. Tru-Cut biopsy devices have the important drawback that they cannot be operated if there is not ca. 1cm of distance between the target of the biopsy and the shell, in the direction of the insertion of the needle. BioPince devices do not need additional space but the existing commercial model has a minimum core length of 13 mm. This device obtains a cylindrical sample of about 7 mg that can be easily extracted by a freezing-thawing process or other extraction techniques. Scallops subjected to biopsies with BioPince and Tru-Cut show relatively low mortality. The coefficient of variation of the biopsies of the digestive gland was found to be between 27 and 41%, depending on the extraction procedure.

PO.05-39
Development of sensitive LCMS methods for the evaluation of excitotoxic amino acids in marine algae.

Session: PO.05 - Toxin analysis

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A novel hydrophilic interaction liquid chromatography mass spectrometry (HILIC-MS) method was developed for the separation of excitatory amino acids (EAA) including kainic acid, domoic acid, glutamic acid, aspartic acid, ß-oxalylamino-L-alanine and L-ß-methylamino-alanine in a variety of marine algae. Several HILIC stationary phases were assessed during this study including TSK-gel amide-80 (Tosoh), Atlantis HILIC silica (Waters) and the highly polar, zwitterionic ZIC HILIC (SeQuant). In each case, a variety of parameters were optimised including buffer concentration, organic modifiers, ion-pairing agents, pH, column temperature and flow rate. Single ion monitoring (SIM) of the positive and negative parent ions was performed using an API III, API 165 and API 4000 mass spectrometer. Positive and negative MS/MS spectra were obtained for each EAA examined with the API4000 triple quadrupole MS which facilitated optimization of collision energy and declustering potentials of major fragment ions for single and multiple reaction monitoring. All aspects of quantification will be discussed including linearity assessment, level of detection, level of quantification and effects of sample matrix for each EAA under investigation. These studies were applied to purity assessment of algal derived products such as kainic and domoic acid, and excitotoxin identification in seaweeds, diatoms and cyanobacteria.

PO.04-09
The uptake of domoic acid by jellyfish: a new phycotoxin vector?

Session: PO.04 – Food chains

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The trophic transfer of domoic acid (DA) through marine food webs is well-established. Many vectors have been identified that either consume directly DA-producing *Pseudo-nitzschia* or acquire the toxin indirectly. A jellyfish mortality event was associated with a *Pseudo-nitzschia* bloom and dissolved DA levels exceeding 120 nM in Monterey Bay, USA, indicating the possible uptake of DA by jellyfish. This suggests that jellyfish can serve as vectors for transferring toxin to its predator species (e.g., turtles, dolphins, tuna) in this and other locations. Laboratory experiments using the jellyfish *Aurelia aurita* were performed to assess toxicity (LD50 96 h) of dissolved DA and the ability of these organisms to accumulate the toxin, with toxin levels measured using a receptor binding assay. Results demonstrated that DA was not toxic to *A. aurita* at the concentrations tested (3 to 250 ng/mL) over a 96 h exposure. During the same time period, a linear relationship was observed between the DA level in seawater and in the jellyfish, suggesting diffusion as the primary uptake mechanism. These findings demonstrate that jellyfish can accumulate DA and thus serve as vectors for trophic transfer and geographic transport of the toxin as these contaminated organisms are moved by ocean currents.

PO.05-31
The study of cryptic PSP toxicity depending upon the extraction procedure

Session: PO.05 - Toxin analysis

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The recent modifications of the extraction solvent for pre-chromatographic oxidation LC method of Lawrence *et al*. (2004) were tested with shellfish harvested during a bloom of *Gymnodinium catenatum* that occurred along the NW coast of Portugal during autumn 2005. The toxic profile in several shellfish species was obtained using acetic acid extraction, and compared with hydrochloric acid extraction. Since the acetic acid does not promote the partial conversion of N-sulfocarbamoyl analogues, as observed with 0.1M hydrochloric acid, underestimation of carbamate toxins was recorded. A more complete conversion of N-sulfocarbamoyl toxins was achieved with 1 M hydrochloric acid, meaning cryptic toxicity was still left in the sample.

PO.01-01
Genetic characterization of *Pseudo-nitzschia* species isolated from the Chesapeake Bay, Maryland USA

Session: PO.01 - Genetics

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Recently, domoic acid-producing *Pseudo-nitzschia* species were isolated from the Chesapeake Bay, Maryland (see poster by A. Thessen, this meeting). Although *Pseudo-nitzschia* has been identified previously in this region via light microscopy, there has been very little work performed to identify them via electron microscopy, genetics and toxin analyses. As part of the characterization of these isolates, we sequenced two ribosomal RNA loci: the 28S large subunit and the region spanning internal transcribed spacer 1, 5.8S, and internal transcribed spacer 2. Sequencing of both loci confirmed the electron microscopy identification of one *P. multiseries* culture, five *P. fraudulenta* cultures and seven *P. calliantha* cultures. These sequences were identical, or nearly identical, to sequences available on GenBank derived from European and US west coast isolates. This work confirms the presence of toxic *Pseudo-nitzschia* in the Chesapeake Bay and preliminarily demonstrates that we are detecting some of the same strains that have led to marine mammal deaths and human illness in other parts of the world.

PO.15-18

Rapid field-based monitoring systems for the detection of toxic cyanobacteria blooms: microcystin immunostrips and fluorescence-based monitoring systems

Session: PO.15 - Monitoring

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The occurrence of toxic cyanobacteria is an increasing problem as the world places more demand on its water supplies. Current monitoring strategies are man-power intensive. Discrete samples are collected and returned to the laboratory for chemical or biochemical analysis. This approach is slow and difficult to implement in less-developed regions. Here we describe two alternative approaches for monitoring cyanobacterial blooms and their toxins. The first uses the fluorescence of the cyanobacterial pigment phycocyanin. Dual fluorometers were installed in a research vessel and used to monitor for chlorophyll and phycocyanin-containing blooms while the ship was underway. Results of the autonomous ship-board monitoring from the great lakes, as well as application of a simpler hand held unit will be discussed. In a second approach, a lateral flow immunoassay (ImmunoStrip) was constructed for the hepatotoxin, microcystin-LR. This ImmunoStrip could detect soluble microcystins at concentrations below 10 ug per liter. Coupled with a field extraction technique, the ImmunoStrip could quickly determine if a toxic bloom was present without returning the samples to the laboratory. Comparison of this assay with other techniques will be presented.

PO.06-16

***Alexandrium minutum* and *Kryptoperidinium foliaceum* blooms in different environmental conditions in the Miñor River influenced region (NW of Spain)**

Session: PO.06 - Population dynamics

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Baiona Bay was selected to study the spring and summer bloom dynamics of *Alexandrium minutum* since this area is frequently affected by its toxicity while nearby areas are usually free of it. The zone is affected by the tidally intermittent freshwater discharge from Miñor

River at the SW part of the bay and by local breeze.
The researched area ranged from this part of the bay, the estuary and the Miñor River up. A *A. minutum* bloom was detected on 18 August in the middle of the south part of the bay during low tide, reaching up to $8 \cdot 10^5$ cells·L^{-1}, associated with salinities and temperatures from 35.3 to 35.7 psu and 17 ºC to 18.5 ºC, respectively. Nevertheless this bloom was not observed the following day or the next week. Moreover, during the same month a *Kryptoperidinium foliaceum* bloom was observed in both the estuary and in Miñor River (2-3 km up). This species was associated with the estuarine front formed by the surface freshwater outflow and the entrance of higher density seawater. It is discussed that differences in the life cycle of the two species may be related to the different behaviour and physical conditions associated with their blooms.

PO.13-66

A decade monitoring toxic phytoplankton in Scottish waters

Session: PO.13 - Regional events

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A programme, monitoring the presence of potential shellfish toxin producing phytoplankton species, in fulfilment of 91/492/EEC, was operated by FRS from 1996 to 2005. This programme identified a number of regional hotspots for the presence of *Alexandrium* cells along the Scottish coast. Over the monitoring period, decreased *Alexandrium* cell densities were recorded during early summer, correlating with lower PSP toxin concentrations in farmed mussels (*Mytilus edulis*). *Dinophysis acuminata* and *D. acuta* dominate the *Dinophysis* population in Scottish waters. A change in the ratio of abundance of these species has been recorded since 2001. Elevated cell densities of *D. acuta* were associated with increased closures of cultivated mussels inferring that this species is more toxic. Since 1999, extensive closures have been enforced in offshore scallop fishing grounds due to high concentrations of ASP toxins in king scallops (*Pecten maximus*). Transmission electron microscopy of *Pseudo-nitzschia* species in Scottish waters has revealed diverse populations. In total more then ten species were identified. Changes in the occurrence of toxin producing phytoplankton species in Scottish waters will be discussed.

PO.04-07

A test of toxic vs. nutritional effects of harmful algae (brown tide) on clam larvae and implications for benthic recruitment

Session: PO.04 – Food chains

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Brown tides of *Aureococcus anophagefferens* coincide with summer planktotrophic larval development of the commercially important bivalve, *Mercenaria mercenaria*, in mid-Atlantic USA. This study investigates effects of toxic and non-toxic *A. anophagefferens* isolates on larval growth, survival and dynamics of

lipid classes and fatty acids. The toxic strain consistently inhibited growth in a dose-dependent manner, relative to an *Isochrysis galbana* control, leading to arrested development in D-stage, but yielded variable mortalities. Larvae did not recover when returned to the control diet after 2 wk-exposure to unialgal brown tide; 20% of the population recovered following exposure to an *A. anophagefferens*/*Isochrysis galbana* mixture, suggesting genetic variability in susceptibility to brown tide. Non-toxic *Aureococcus* supported growth rates comparable to *I. galbana*. Larvae exhibited reduced filtration on unialgal brown tide and selected against toxic cells in mixed suspensions, as confirmed by gut autofluorescence and flow cytometry. Experimental approaches allowing discrimination between algal toxicity and nutritional deficiency are evaluated. We conclude that brown tides will cause metamorphic failure of clam larval populations primarily through toxin-mediated, growth inhibition. Prolonged residence time in the plankton will increase vulnerability to secondary mortality factors. In turn, hard clam larvae likely make a negligible contribution to microzooplankton grazing on brown tide.

PO.02-09
Mating incompatibility: genotyping of *Alexandrium* cysts

Session: Genomics

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Though the ranges of toxic and non-toxic strains of the dinoflagellate *Alexandrium tamarense* are not thought to overlap, these cell types do occur in close geographic proximity. Unpublished data (D. M. Anderson) show that toxic and non-toxic strains of this species can fuse sexually to form resting cysts (hypnozygotes). However, all hybrid hypnozygotes die shortly after germination. Such post-zygotic lethality (PZL) may be a barrier to range expansion of toxic blooms. It is not yet known whether such hybrid fusions occur in nature; toxic cells may fuse only with other toxic cells if allowed a choice of compatible toxic and non-toxic types. Here, a PCR method for analysis of cysts resulting from competitive crosses of toxic and non-toxic strains is described. With this method, it is possible to differentiate hypnozygotes arising from two toxic, two non-toxic, and toxic/non-toxic parents. Analysis of single hypnozygotes and pellicle (non-zygotic) cysts, in conjunction with previously published data, suggests that pellicle cysts may be incompletely transformed zygotes rather than haploid cells. A remaining obstacle to reliable observation of mating selectivity is the lack of a rapid and penetrant method for sexual induction of *A. tamarense* in culture.

PO.13-62
Seasonal diversity of *Pseudo-nitzschia* species in the Shetland Isles, Scotland

Session: PO.13 - Regional events

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Members of the genus *Pseudo-nitzschia* have been associated with extensive closures of offshore Scottish scallop fishing grounds due to the accumulation of high concentrations of domoic acid in the gonad tissue of *Pecten maximus*. Four research cruises during spring and autumn 2004/2005 surveyed the phytoplankton around the Shetland Isles to investigate the diversity of *Pseudo-nitzschia* populations in this area. Light microscopy analysis showed two *Pseudo-nitzschia* morphotypes: (*P. seriata* 'type' cells (diameter >3µm) and *P. delicatissima* 'type' cells (diameter <3µm)) to be present at high cell densities (>500,000 cells l^{-1}). Detailed analysis of these samples using transmission electron microscopy identified a diverse population structure. The species observed included *P.* cf. *seriata*, *P.* cf. *australis*, *P. pungens*, *P. fraudulenta*, *P*. cf. *decipiens* and *P. pseudodelicatissima.*

PO.10-11
Tracking through carbon and nitrogen isotopes if the food ingested by *Prymnesium parvum* is from an animal or a plant

Session: PO.10 - Ecophysiology & autecology

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Understanding pathways of carbon and nitrogen through the aquatic food webs are of fundamental importance in order to understand how these ecosystems works. It has been shown that the use of stable carbon and nitrogen isotope signatures can be used to trace the flow of organic matter through the aquatic food web and to determine trophic position of the organisms involved. The isotope ratios differ between organisms and their diets because of slight selective retention of the heavy isotope and excretion of the lighter isotope. As a result, organisms tend to become enriched by heavy isotopes as compared to their diet. Despite the fact that stable isotopes are increasingly being used, few experimental studies have addressed the relationship between the isotopic composition of mixotrophic species and their diets. The aim of our study was to quantify the relative importance of photosynthesis vs. phagotrophy in *Prymnesium parvum* when fed an animal (*Oxyrrhis marina*) and a plant (*Rhodomonas* sp.) using stable isotopes during a 12-h experiment. *Prymnesium parvum* was cultured under nitrogen deficient, phosphorus deficient and in nutrient sufficient conditions. The experiment was performed in light and complete darkness. Results from the stable carbon and nitrogen isotope signatures will be discussed.

PO.13-16
Blooms of *Aphanizomenon flos-aquae* associated with historical trophic changes in Swietokrzyskie Lake, Poland

Session: PO.13 - Regional events

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Sediment records from 7570 +/- 150 BP in Swietokrzyskie Lake have indicated increased trophic status to present hypertrophic conditions. High values of phytoplankton biomass in the lake (82.0-611.7

mg/dm^3) have been associated with increased concentrations of ammonia nitrogen (2.5–9.2mg /dcm^3), and organic phosphorus (0.97–3.07 mg/dcm^3), representing a polytrophic status (Burchardt 1987). *Aphanizomenon flos-aquae* surface blooms in the lake were associated with increasing numbers and size of gas vacuoles in their trichomes, and occurred during the initial phase of the bloom and associated with higher iron concentrations (e.g. 0.18 mg/l). Vegetative cell division of *A. flos-aquae* and production of heterocysts and akinetes occurred when phosphate levels exceeded 0.3 mg/l (Burchardt 1987). The lake water analysis in 1998 included the concentration of algal biomass, nutrients, N:P coefficient, and iron (Burchardt, Kokocinski, Owsianny 2000). The combinations of the different mineral forms of iron to mineral forms of nitrogen and phosphate were found to be responsible for inhibiting development of *Aphanizomenon flos-aquae* as well as for the development of akinetes from sediments. Avaibility of the mineral form of iron (III), involved in the photosynthesis process, was found to be a stimulating factor for increased abundance of *Aphanizomenon flos-aquae* in Swietokrzyskie Lake.

PO.01-24
Phytoplankton assemblages in ballast water of U.S. military ships considering port of origin, voyage time and ocean exchange practices

Session: PO.01 - Genetics

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Environmental conditions and phytoplankton assemblages were characterized in ballast water from 62 ballast tanks aboard 28 ships operated by the U.S. Military Sealift Command and the Maritime Administration, sampled at 9 ports on the U.S. West Coast and 4 ports on the U.S. East Coast. The ballast tank waters had been held for 2-176 days, and 90% of the tanks had undergone ballast exchange with open ocean waters. One hundred phytoplankton species were identified including 22 potentially harmful taxa. Assemblages were dominated by chain-forming diatoms and dinoflagellates, and viable organisms comprised about half of the total cells. Phytoplankton abundances were unrelated to the measured physical/chemical parameters, except for a positive relationship between centric diatom abundance and nitrate concentrations. Abundance generally was higher in tanks with coastal water sources and decreased with water age. Tanks with ballast water more than 33 days old did not produce culturable phytoplankton. Abundances of viable phytoplankton with maximum dimension > 50 µm exceeded proposed International Maritime Organization standards in 47% of the ballast tanks sampled. The data suggest that further treatment

technologies and/or alternative management strategies will be necessary to enable DoD vessels to comply with proposed standards.

PO.04-06
Lethality of microalgae to farmed Atlantic salmon

Session: PO.04 – Food chains

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Blooms of phytoplankton in the Bay of Fundy have been implicated in the deaths of farmed Atlantic salmon. To establish whether or not elevated concentrations of these algae can cause mortality, monocultures of two species of microalgae, *Alexandrium fundyense* and *Ditylum brightwellii* were grown in large quantities. Atlantic salmon smolts were exposed to a range of concentrations of these cultures for 24 h and an LC50 was determined according to the concentration of cells present (cells/L). Fish exposed to *Ditylum brightwellii* at concentrations as high as 10^6 cells/L had no apparent deleterious effect. This concentration is well above the concentration observed in the field. Salmon exposed to *A. fundyense* were affected. The LC50 was estimated to be ~300,000 cells/L - a concentration that has been observed in the field. *Alexandrium fundyense* is a known neurotoxin producer and is the organism responsible for causing paralytic shellfish poisoning. Work is continuing to determine if other species found in the Bay of Fundy can cause problems for cultured fish and to determine the concentrations of toxins in the *Alexandrium* cultures and fish tissues.

PO.08-27
Variations in growth and toxicity of *Gymnodinium catenatum* from the Gulf of California under several ratios of nitrogen and phosphorus

Session: PO.08 - Toxicology

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One anthropogenic influence on the coastal waters is the input of nutrients, which is reflected in the increase and selectivity of particular autotrophic organisms. *Gymnodinium catenatum* Graham is a PSP producer, widely distributed along the Pacific coast of Mexico, linked to poisoning events in coastal waters, as well as poisoning of shellfish consumed locally. In this study, *G. catenatum* was submitted to several nutrient scenarios to measure its growth and toxin production. The greatest density occurred at the 16:1 ratio, producing 33% more cells than the 32:1 and 64:1 ratios, which, in turn had 34 and 30% more cells than the 1.6:1 and 3.2:1 ratios. Total toxicity was not affected by the treatments but depended on the growth phase. In general, toxicity decreased slightly over time, but increased at the end of the decay phase. The toxin profile was dominated by sulfocarbamoyl toxins (C types) with about 60 to 70% on a molar basis, followed by the carbamoyl toxins with about 5 to 25% on a molar basis. These data suggest that changes N:P ratios will not stimulate toxicity production, but will mainly effect the growth of *G. catenatum*.

PO.10-08
Enhanced growth of *Heterosigma akashiwo* at high light intensity

Session: PO.10 - Ecophysiology & autecology

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Blooms of the raphidophyte *Heterosigma akashiwo* occur every year in the Nervion River Estuary, coinciding with calm weather and high irradiance. To examine whether this alga benefits from high levels of irradiance without experiencing apparent photoinhibition, two strains isolated from the estuary were cultured at irradiance levels ranging from 100 to 1200 micromol quanta m^{-2} s^{-1} in a culturing medium prepared with estuarine water covering a broad range of salinities. The temperature in the culture medium was maintained at 20 ºC and the photoperiod was 12:12 h light:darkness. The growth rates of both strains increased with light intensity up to an irradiance of 350 micromol quanta m^{-2} s^{-1} after which the growth rates remained nearly constant but without showing any appreciable photoinhibition. Growth rate values at high light intensities were of the order of 1 divisions per day even at 1200 micromol quanta m^{-2} s^{-1}, which is a common rate for this species. We hypothesize that the ability of *Heterosigma* to migrate in the water column and the elevated growth rates this algae is able to maintain at extraordinarily high irradiances, makes it a strong competitor in the surface waters of the estuary on calm and sunny days.

PO.15-20
Spatiotemporal data mining for tracking harmful algal blooms

Session: PO.15 - Monitoring

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In this paper, we present a spatiotemporal data mining model for tracking and predicting the movement of Harmful Algal Blooms (HABs) from satellite image database SeaWiFS and field cell counts. We found that the spatial density filtering algorithm shows promise in processing of the image data. The mutual information model is able to track the target based on the spatial patterns over time, when the shape is coherent. With a defined environment and enough training data, the machine learning model potentially is capable of predicting a short-term spatial dynamics. The challenge ahead is how to combine data mining models with multi-physics models.

PO.08-14
Evaluation of the toxicity of *Prorocentrum* species by liquid chromatography-mass spectrometry and cell-based assay

Session: PO.08 - Toxicology

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Dinoflagellates belonging to the genus *Prorocentrum* collected in Sabah, East Malaysia (*P. rhathymum*, *P*. cf. *faustiae* and three strains of *P. lima*), La Réunion, (*P. belizeanum*) and in Alfacs Bay and Vigo, Spain (*P.* cf. *mexicanum*, *P. lima*) were cultured to study their toxicological and toxin production properties, by cell assays and liquid chromatographic methods, using either fluorescence or mass-spectrometry detection (LC-FD, LC-MS). Cytotoxicity, characterised by IC_{50} and morphological changes, is studied and compared.
IC_{50} ranged from 2103 cell equivalents for *P*. cf. *faustiae* to 133,103 for *P. rhathymum*. Morphological changes such as rounding and membrane blebbing, observed in cells exposed to all *Prorocentrum lima* from Malaysia and to *Prorocentrum belizeanum*, suggest the presence of okadaic acid (OA). Analytical measurements confirmed the production of OA by these species. However, toxicity not correlated with the presence of OA suggests the production of derivates of OA or other toxins.

PO.07-10
Convergent blooms of *Karenia brevis* along the Texas coast

Session: PO.07 - Ecology and oceanography

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A numerical model of wind-driven surface flow in the Gulf of Mexico is used to examine physical controls on harmful algal bloom formation along the Texas coast. *Karenia brevis*, which blooms sporadically throughout the Gulf of Mexico, has a relatively slow growth rate (doubling times of 2-3 days). Increases in *K. brevis* concentration cannot be explained simply in terms of growth. We hypothesize that the primary mechanism responsible for bloom formation is convergence due to downwelling at the coast. Convergence along the Texas coast caused by seasonal downwelling winds can concentrate the plankton up to 1000 times. This is surprising because the modelled cells do not grow; the simulated increase in concentration is due to physical processes alone. The numerical model does a reasonably good job at predicting the timing and magnitude of bloom initiation along the coast, but does not predict the details of the migration of the bloom along the coast after it has formed, or the destruction of the bloom. This result is significant because it implies that *K. brevis* blooms may be caused primarily by physical processes, and that cell division is not an important factor in bloom formation.

PO.13-46
Dinophysis sacculus from Alfacs Bay, NW Mediterranean. Toxin profiles and cytotoxic potential

Session: PO.13 - Regional events

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Dinophysis sacculus is commonly present at the Mediterranean and

Atlantic European coasts. It is the principal cause of diarrhetic shellfish poisoning (DSP) toxic episodes in a seafood production area in the Ebro Delta, Spain.This species has been found to produce okadaic acid (Delgado *et al*. 1996). As for other species of the genus *Dinophysis*, it is not yet possible to obtain cultures that would facilitate its study.
A bloom of *D. sacculus* was detected in a small pond connected to Alfacs Bay during the fall of 2005 and winter of 2006. This has allowed us to make an estimation of the toxin profile of this species in this area. In some samples collected within this period, *D. sacculus* represented more than the 90% of the phytoplankton population with concentrations up to 81600 cells/L. Cytotoxic assays and analytical procedures were used to identify the toxins and the toxin potency of this species. The toxicity of this species was evaluated with cell viability (MTT assay), IC_{50} was determined as well as the morphological changes. LC-MS analysis allowed the detection of PTX2, PTX2sa and okadaic acid.

PO.14-10
Removal of red tide organisms by organo-clays: removal mechanisms and ecological effects

Session: PO.14 - Mitigation

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Several organo-clays were prepared by modification of clays with different quaternary ammonium compounds (QACs). The modifications influenced the capacity of the organo-clays to remove red tide organisms. The removal mechanisms and ecological effects are discussed. Hexadecyltrimethylammonium (HDTMA) bromide improved the capacities of clays to flocculate the algae. One of the reasons was that HDTMA in metastable state was toxic to the algae. The influence of the organo-clays on nutrients, DO and COD in seawater was also studied. The organo-clays had better capacity to adsorb nutrients, especially phosphate, than the original clays. Seawater parameters, such as DO, COD and pH also improved after treatment of harmful algal blooms by the organo-clays. The clays not only controlled HABs effectively, but also benefited seawater quality. Experiments in a co-culture system indicated that the red tide organisms (*Heterosigma akashiwo*, *Prorocentrum donghaiense*) could be efficiently extinguished without any evident impact on co-cultured organisms such as *Crassostrea gigas*.

PO.16-14
Sexual compatibility assays in *Pseudo-nitzschia* species from the Aveiro coastal lagoon (NW Portugal)

Session: PO.16 – Life cycles

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Portuguese isolates of the following five *Pseudo-nitzschia* species were mixed and tested for the capacity to mate and produce auxospores: *Pseudo-nitzschia americana*, *P. australis*, *P. fraudulenta, P. multistriata* and *P. pungens*. Additionally, sexual compatibility was assayed with strains from St. Lawrence, Canada (*P. fraudulenta*), Denmark (*P. pungens*, *P. seriata*), Norway (*P. obtusa*), Ukraine (*P. pungens*) and Washington State, USA (*P. australis, P. pungens*). Mating and auxospore formation was only found in *P. pungens*. Among 10 Portuguese strains of *P. pungens*, only four were involved in mating and auxospore formation; one of the strains being involved in all matings represents one mating type. Although the Portuguese strains showed no sexual compatibility with strains from other regions, four succesfull matings among the latter were observed. these involved four strains from Ukraine, none of which was common to all successful crosses. One of the American strains produced auxospores with a Ukrainian strain. In all successful matings, sexual interaction was usually detectable one day after mixing the strains and the main events of gamete formation and fusion occurred during late evening and night. Auxospore growth continued for an average of about 30 h.

PO.10-34

RNA content and growth rates in *Alexandrium* species cultured under varying environmental conditions

Session: PO.10 - Ecophysiology & Autecology

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Three cultures of *Alexandrium*, *A. minutum*, *A. ostenfeldii* and a North American strain of *A. tamarense*, have been studied under various conditions, including salinities ranging from 15 psu to 40 psu and temperatures from 15 °C to 25 °C, and growth curves have been produced. Using these growth curves the specific growth rate has been calculated for these strains/species under each environmental condition and thus the optimal, maximal and minimum growth rates for each have been determined. Cells have been harvested from different growth phases and under differing growth conditions and the RNA concentration per cell measured. These studies are being undertaken to validate the quantitative use of probes that have been designed to identify individual species of *Alexandrium* (and other nuisance species of algae) in waters with different environmental conditions. These probes will form an integral part of an RNA biosensor that is currently being developed within the EU supported Algadec project.

PO.10-09

Mixotrophy in *Dinophysis norvegica* populations in natural communities occurring in the Baltic Sea

Session: PO.10 - Ecophysiology & autecology

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Dinophysis norvegica is known to form sub-surface blooms, produce toxins and to ingest particulate organic matter. Although phagotrophy of photosynthetic *Dinophysis* species is not a new discovery, its role in the ecology of *Dinophysis* spp. is still unclear. We used flow cytometry and an acidotropic probe to quantify the frequency of *D. norvegica* cells containing food vacuoles occurring in natural populations collected from the Baltic Sea. At the beginning of the bloom (June 29th 2004), 27.43 % of the *D. norvegica* cells contained food vacuoles. A month latter (July 27th), 49.67 % of the *D. norvegica* cells in the whole water column had food vacuoles, the water column was well stratified and nitrogen limited. The highest cell density (341 cells/l) was recorded in the aphotic zone (30 m depth), where 71% of the *D. norvegica* cells contained food vacuoles. Real-time PCR assay and microscope observations showed that part of the population (5%) was in a late stage of cell division. These results suggest that phagotrophy was the main nutritional pathway for *D. norvegica*, supporting the formation and maintenance of sub-surface blooms of *D. norvegica* in the Baltic Sea during the summer.

PO.13-15
Didymosphenia geminata: a new invasive diatom

Session: PO.13 – Regional events

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The freshwater benthic diatom *Didymosphenia geminata* (Lyngbye) Schmidt (*Didymo*) is emerging as an organism with an extraordinary capacity to impact stream ecosystems. This stalked diatom is able to dominate stream benthos by covering 100% of substrates with thicknesses of up to 20 cm, greatly altering physical and biological conditions within streams. A paradox exists in that these dense blooms occur in streams that would otherwise be considered extremely oligotrophic. In recent years, streams and rivers in New Zealand, North America, Europe, and Asia have been colonized by unprecedented masses of *Didymo* and its extra cellular stalks. The pattern of expansion of the geographic range of *Didymo* blooms around the globe suggests that human activity is an important vector. Although nuisance blooms of *Didymo* are increasingly reported by the public and local media there has been little scientific investigation of the phenomenon outside of New Zealand. Recent work on the global distribution, ecology, genetics and on-going mitigation efforts will be presented.

PO.07-07
Control of toxic algal bloom by a tiny parasitoid

Session: PO.07 - Ecology and oceanography

A Chambouvet, L Guillou

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Since 1988, an invasive harmful blooming species *Alexandrium minutum* Halim has been repeatedly recorded along the Atlantic French coast in the Penzé Estuary. First detected as massive toxic blooms during the first years of occurrence (about 10^7 cells/l), this species is still detected every year, but it no longer foms blooms (about 10^5 cells/l). At the same time, two novel eukaryotic lineages belonging to the Alveolata (Group I and Group II) were detected by culture independent methods (genetic diversity from total genomic DNA) collected from a large array of

marine ecosystems (sea surface to marine hydrothermal ecosystems). We used a specific oligonucleotide probe that target the whole group II to detect these organisms by fluorescent *in situ* hybridization during the growth of *A. minutum* in the Penzé Estuary during two consecutive years. Members of this group are efficient parasitoids of dinoflagellates and their occurrence was apparently tied to the termination and demise of *A. minutum* blooms. Bsed on the preferential amplification of the SSU rDNA, almost all were closely related to the wellknown dinoflagellate parasitoid *Amoebophrya* spp. (ex ceratii).

PO.06-27
FINAL, an interreg program for forecasting the initiation of toxic algal blooms

Session: PO.06 – Population dynamics

Annie Chapelle[1], R Raine[2], K. Davidson[3], C. Labry[1]
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Increasing toxic events threaten the economy linked to aquaculture and tourism as well as human health. France, Ireland, Scotland will create a network to work on PSP events due to *Alexandrium* and ASP events due to *Pseudo-nitzschia*.
The objectives are to investigate environmental factors linked to the occurrence of toxic events and to propose an alert based on these indicators, which can be obtained by monitoring by weather forecast and by environmental modelling.

PO.01-09
Development of a molecular probe for the harmful alga, *Pyrodinium bahamense* var. *compressum*, from Sabah, Malaysia

Session: PO.01 - Genetics

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University Malaysia Sabah, KOTA KINABALU, Malaysia

Harmful algae blooms in Sabah only occur in the coastal waters of west Sabah, where one of the causative organisms is the dinoflagellate, *Pyrodinium bahamense* var. *compressum*. *Pyrodinium* blooms are often unpredictable and sometimes occur at very low densities. One of the problems in the monitoring and management of the *Pyrodinium* red tides is the early detection of the species. This study involves the development of a DNA probe from highly conserved small subunit rDNA regions for fast and accurate identification of the alga. Seawater samples containing *Pyrodinium* taken from four offshore locations were isolated and cultured in f/2 medium. After DNA extraction, PCR amplification was carried out targeting the rDNA of the algae. The results of eight restriction enzyme digestions indicated that all the cultures were similar. Sequences of other *Pyrodinium* isolates were also obtained (Accession number: DQ500119 - 23). The size of the sequences ranged from between 1580 to 1544 bp. They differed from the sequence of *Pyrodinium bahamense* from the GenBank in 1-4 nucleotide bases.

PO.12-06
FITC-conjugated lectins as a tool for differentiating between various Polynesian strains of the ciguatera-causing dinoflagellate, *Gambierdiscus* spp.

Session: PO.12 - Taxonomy and phylogeny

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The benthic dinoflagellate *Gambierdiscus* spp. is the primary causative agent of Ciguatera Fish Poisoning (CFP). Currently, 6 distinct species have been described within this genus. Evidence for the existence of toxic and non-toxic clones within a given species is also documented in the literature. The coexistence within natural populations and seasonal blooms of multiple species of *Gambierdiscus*, comprising both toxic and non-toxic clones, is often speculated, stressing the need for (molecular) tools capable of differentiating between the various strains of this dinoflagellate.
We compared the binding patterns of 11 FITC-conjugated commercial lectins - *Dolichus biflorus* (DBA), *Canavalia ensiformis* (Con A), *Triticum vulgaris* (WGA), *Pisum sativum* (PEA), *Arachis hypogaea* (PNA), *Helix pomatia* (HPA), *Ulex europaeus* (UEA), *P. tetragonolobus* (PTG), *B. simplicifolia* (BS-1), *L. esculentum* (LEA) and *Lens culinaris* (LcH) - between various clones of *G. toxicus*, *G. polynesiensis*, *G. australes* and *G. pacificus* isolated from French Polynesia.
Lectins appeared to be an interesting tool to identify and differentiate between the different species of *Gambierdiscus*. Furthermore, some lectins were also able to discriminate between toxic and non-toxic clones.
The potential use of the molecules for detection and characterization of harmful *Gambierdiscus* blooms in nature is discussed.

PO.09-04
Genetic characteristics of non-toxic subclones obtained from toxic clonal culture strain of *Alexandrium tamarense* (Dinophyceae)

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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This study was conducted using subclones of the PST-producing dinoflagellate *A. tamarense* obtained from an extremely rare clonal culture strain (OF935-AT6), which was found to have become a mixture of toxic and nontoxic cells during maintenance. The nontoxic characteristics of UAT-014-009, an axenic nontoxic subclone of OF935-AT6, were confirmed by three different methods. Both nontoxic and toxic subclones from OF935-AT6 were confirmed phylogenetically by nucleotide sequence analysis of species-specific regions in rDNAs to be the common *A. tamarense* found around Japan. Three out of nine toxic subclones were found to have lost toxicity during a short period of time (four or six years), whereas the other toxic subclones retained their toxicity within the same range as formerly observed. The subclones of OF-935-AT6 showed a variety of AFLP patterns targeting mitochondrial genes (cytochrome c oxidase and 5'-flanking region of cytochrome b), whereas re-isolated subclones (sub-subclones) from the subclones of OF-935-AT6 showed homogenous toxicity and AFLP genotypes. These results suggest that the parent OF935-AT6 culture

had become very heterogeneous in terms of toxicity and mitochondrial genotypes; and the stability of them, although the OF935-AT6 was established from one single cell from natural population.

PO.02-08
Mononucleotide polymorphism of a microcystin synthetase, mcyH in the releasing of microcystins in a specific strain of *Microcystis aeruginosa*

Session: PO.02 - Genomics

HN Chou
National Taiwan University, TAIPEI, Taiwan

Microcystins are a group of hepatotoxins produced mainly by toxic species of *Microcystis* through the function of microcystin synthetases that are coded from a series of genes, mcyA~mcyJ. The toxins are produced within the cyanobacteria cells and are expected to be predominantly found in slow-growing, healthy cells. Microcystin release is generally considered to be linked to a decrease in the integrity of the cells. However, in a strain of *M. aeruginosa* it was found microcystins are released during the log phase of growth and continuously accumulate in the cell-free medium. A single nucleotide difference of the sequence mcyH was found in the toxin-releasing strain compared to other non-toxin releasing strains. The sequence of mcyH is known to be homologous to the bacteria transporter, but is also known as a key protein that maintains the integrity of the microcystin synthetase clusters.

PO.08-02
Biologically active substances with spiro-linked rings in seafood

Session: PO.08 - Toxicology

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In the middle of the 1990s a new class of neurotoxins was discovered in aquaculture sites along the East Coast of Canada. These toxins were found to be 'fast acting' toxins causing death within several minutes when injected into mice. The molecular structure of the toxins, named spirolides, consists of a spiro-linked, tricyclic system of polyethers and a seven-membered spiro-linked cyclic iminium moiety. Spirolides were first isolated from shellfish and later from plankton samples. In 2000, *Alexandrium ostenfeldii* was identified as the causative organism of spirolides. Whereas spirolides belonging to groups A-D are biologically active, spirolides E and F show no biological activity. The instability of the cyclic iminium function under enzymatic or acid conditions can be important with respect to an oral toxicity of spirolides.
The Alfred-Wegener-Institute for Polar- and Marine Research (AWI) on Helgoland Island succeeded in cultivating a Danish strain of *Alexandrium ostenfeldii* (KO287). Subsequently the extracts were tested for spirolides by LC-MS/MS. For a study of the metabolisation of spirolides in shellfish, extracts from tissues of edible mussels were incubated with an extract of KO287. The measurement of extracts from mussels´ tissues revealed no characteristic differences in the spirolide profile compared to KO287.

PO.05-46
Protease inhibition assay as a tool to test the toxicity of cyanobacterial toxins

Session: PO.05: Toxin analysis

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Bioactive compounds from cyanobacteria can affect other biota and the adverse effects on e.g. *Daphnia* survival and feeding have often been used as a first approximation of toxicity. Recently, it has been reported that toxins such as microcystin and microviridin can have strong protease inhibitory effects on e.g., trypsin, chymotrypsin and elastase. This study made *in vitro* test of mixed solutions of extracted cyanobacterial metabolites from cultures and lake scums as well as purified peptides on daphnid proteases. Trypsin-like proteases were extracted from *Daphnia magna*. The effects of metabolites from cultured strains *Microcystis* and *Planktothrix* and from mixed lake bloom material were tested on the daphnid trypsin-like enzyme activity using a photometrical detection. The majority of the extracts tested were inhibitors of daphnid trypsin-like enzymes as well as on the pork trypsin used for comparison. In conclusion, protease inhibitors which occur in many laboratory strains and field populations of *Microcystis*, *Planktothrix*, and other genera of cyanobacteria may induce severe protease inhibition in *Daphnia*. Thus, protease inhibitors are a potential threat to organisms that deliberately or accidentally take up cyanobacterial cells. The associated physiological implications can include incomplete protein digestion, molting problems, reduced feeding, growth and reproduction as well as death.

PO.04-10
Effects of the toxic dinoflagellate *Alexandrium minutum*, grown under different N/P ratios, on the copepod *Acartia tonsa*

Session: PO.04 – Food chains

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An experimental study was carried out to test the effects of *Alexandrium minutum*, cultured at three different N/P ratios (5, 16 and 80), on the copepod grazer *Acartia tonsa*. The ingestion rate, egg production and hatching success, as well as the toxin content in tissues, eggs and pellets of the copepods were checked at different concentrations of food, which also oncluded a constant concentration of the non-toxic dinoflagellate *Prorocentrum micans*. Cell toxicity of the *A. minutum* varied among the different N/P treatments, but it remained constant during each experiment. Copepods ingested both toxic and non-toxic food at all treatments and concentrations. There was a positive correlation between *A. minutum* concentration and ingestion by copepods. Production of eggs and pellets appeared to be affected by food toxicity, while increasing toxin ingestion reduced hatching success as well as increased toxin levels in tissues and pellets. Our results indicate that *A. minutum* toxicity

affects the reproduction and excretion but not the feeding behaviour of copepods.

PO.13-70
Potentially toxic algal species in Ologe Lagoon, Nigeria

Session: PO.13 - Regional events

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Lagos State University, LAGOS, Nigeria

The environmental factors and phytoplankton population of Ologe Lagoon, Nigeria were studied between January 2000 and December 2001. Seasonal and spatial variations were recorded in water temperature (27.6 ± 5.05 °C), salinity (0.17 ± 0.12ppm) dissolved oxygen (5.7 ± 1.45 mg/l), transparency (0.61± 0.21m) and pH (6.8 ± 0.69). Nutrient inputs from a nearby industrial estate resulted in higher nitrate-nitrogen, sulphate and phosphate concentrations in the high insolation-low rainfall dry season. There were periodic blooms of potentially harmful algal species, including diatoms (*Chaetoceros* & *Nitzschia* sp), blue-green algae (*Aphanizomenon*, *Anabaena* & *Microcystis* spp.) and dinoflagellates (*Prorocentrum lima*, *P. micans*, *Ceratium* spp. and *Peridinium quinquanon*).
The most abundant species were centric diatoms (76.12%) and blue-green algae (11.95%) while species diversity was greater among the green algae, dinoflagellates and pennate diatoms (H = 3.30, 3.27, 3.14 respectively). The blue-green algae and centric diatoms showed lowest diversity (H = 1.51, 1.43). The Berger-Parker Dominance Index (BPDI) showed that the Cyanophyta were dominant while diatoms, green algae and dinoflagellates appeared as sub-dominants during blooms.
The major HAB species in the lagoon showed a patchy distribution with high densities of short duration, probably in response to nutrient inputs from the surrounding environment.

PO.13-21
Harmful microalgae along the Latium coasts (Middle Tyrrhenian Sea, Mediterranean Sea): bloom and toxicity events since 1997

Session: PO.13 - Regional events

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In the framework of a research collaboration with the Environmental Agency of Latium (Middle Tyrrhenian Sea, Mediterranean Sea) the structure and dynamics of marine phytoplankton was assessed at six coastal stations from 1997 to the present day. Past identifications and distributions were updated during a bimonthly monitoring program of harmful populations in water and net samples. Recurrent summer blooms of the dinoflagellates *Ostreopsis ovata* and *Coolia monotis* in rocky, sheltered southern environments also prompted monitoring of microphytobenthos from 2003. A variety of potentially toxic microalgae have been identified with light and electron microscopy. Fifteen thecate and at least five naked dinoflagellates were recorded together with five *Pseudo-nitzschia* species, the latter being responsible for massive annual blooms (up to

10^7 cells/l). There were also sporadic brown tides due to intense growth of *Fibrocapsa japonica* during summer. In bloom periodicity and cell abundance, spring and summer were the most critical periods for toxicity events. In fact, HPLC and electrochemical immuno-sensor analyses indicated the presence of domoic acid in natural phytoplankton populations tested over a full year; using LC-MS/MS methods a palytoxin analogue was also detected in mucous aggregates dominated by *Ostreopsis ovata*.

PO.15-35

Functionalized nanoparticles and dielectrophoretic detection of harmful algae

Session: PO. 15 - Monitoring

Laurie Connel, J. Duy, R. Smith
University of Maine, ORONO, United States of America

A developing trend in harmful algae detection is microfabricated sensors and instruments that utilize electronic sensing mechanisms, which can reduce the size and power requirements, and potentially increase the efficiency of data generation, collection and processing through circuit integration. Water quality managers, researchers and public health officials require timely and accurate detection of harmful species in a cost effective manner. Therefore, there is a need for improved methods capable of rapid on-site analysis. The use of a DNA analog, peptide nucleic acids (PNAs), with their unique binding parameters, is used to decrease target binding time and allow hybridization at very low salt concentrations. HAB specific PNA probes are bound to functionalized gold nanoparticles. The addition of the functionalized nanoparticles to the target solution is used to capture and concentrate target molecules as well as form an electrical bridge in nanotubes. These bridges are then detected via measurement of electrical impedance across the tube and the data collected. We present here our approach and preliminary data.

PO.13-59

A culture collection of harmful marine microalgae in Brazil

Session: PO.13 - Regional events

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The interest for cultivation of microalgae has been increasing in Brazil due to the growth of aquaculture and research on environmental sciences, including tests of ecotoxicology, which reflect a new demand for the evaluation of impact of pollutants. Studies on harmful microalgae are also relevant. After a 75-year phase of descriptive work, including records of algal blooms, since the 1990s Brazilian experts have been also running analytical and experimental studies on harmful microalgae. However, the small number of potentially harmful strains in the ca. 40 Brazilian culture collections of microalgae creates a bottleneck to the development of new approaches for studies on harmful microalgae in the country. The ongoing expansion of the 'Elizabeth Aidar' Microalgal Culture Collection (Fluminense Federal University, Rio de Janeiro State), represents the start of the organisation of a national reference centre for cultivation of marine microalgae. Part of the Collection is dedicated to harmful strains, supplying cultures to researchers countrywide, and contributing to inter-institutitional integration of experts on harmful microalgae. Currently 20 strains of potentially harmful dinoflagellates, raphidophyceans and cyanobacteria are kept in the Collection, and more strains will be incorporated in the near future.

PO.13-82
Domoic acid intrusion into Puget Sound

Session: PO.13 - Regional events
Presentation time: 16:40 - 18:00

FH Cox, Bob Lona, Jerry Borchert

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Domoic acid which causes amnesic shellfish poisoning was detected in razor clams on the Pacific coast of Washington in 1991. Emergency closures of the razor clam and crab fisheries were enacted. As a result, a domoic acid monitoring program was established for the Pacific and Puget Sound fisheries. In 2003, domoic acid was detected inside Puget Sound, at Ft. Flagler State Park. The U.S. Food and Drug Administration action level of 20ppm was exceeded and shellfish closures were initiated. Domoic acid was detected at low levels as far west as Port Angeles, as far east as East Whidbey Island and as far south as Port Ludlow. In October, 2005, domoic acid prompted new closures in Puget Sound, involving Penn Cove and Holmes Harbor, two commercial shellfish areas in the Whidbey Basin. These test results were much higher and impacted shellfish other than mussels. Dungeness crab were also tested but were below the closure level. If domoic acid continues to move into new areas inside Puget Sound, the economic and public health implications would be significant. Puget Sound could be in for some very long shellfish closures, should shellfish like geoducks retain the toxin like razor clams do.

PO.06-17
Molecular approaches to HAB research: who's there and what are they doing?

Session: PO.06 - Population dynamics

KJ Coyne[1], MA Doblin[2], CJ Gobler[3], DA Hutchins[1], SM Handy[1], E Demir[1], KJ Portune[1], SC Cary[1]

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Recent developments in molecular technologies have transformed our approach to HAB research. While increasing accuracy and sensitivity of detection, they also increase sample throughput, making them ideal for routine monitoring of HAB species. Although the technology has moved forward at a rapid pace, few studies have applied molecular methods to move beyond simple monitoring and ask important questions about HAB ecology. A suite of qualitative and quantitative methods are now available to assess spatial and temporal changes in community composition and species-specific responses to environmental perturbation at several taxonomic levels. Here, we present an overview of these methods and their application to research on HAB species in estuarine, freshwater, and ships' ballast tank environments. Using molecular methods, we have investigated factors involved in species selection during bloom initiation, competition for nutrients, vertical migration patterns, cyst germination, species-specific impacts of grazing in natural populations, metabolic gene expression in response to nutrient additions, toxin gene expression during ballast transport and the impacts of toxin gene expression on predation. When combined with an assessment of chemical and physical factors of the environment, these methods provide detailed information about the biotic and abiotic factors that impact the structure of phytoplankton populations in natural environments.

PO.05-38
Certified reference materials for lipophilic toxins

Session: PO.05 - Toxin analysis

S Crain, K Reeves, J Walter, S MacKinnon, P LeBlanc, C Craft, W Hardstaff, N Lewis, M Quilliam

National Research Council, HALIFAX, NS, Canada

The ingestion of shellfish contaminated with toxins can lead to severe symptoms such as diarrhoea, amnesia, paralysis and even death. Routine monitoring is essential for public health and for international trade. Regulatory testing requires validated methods and a quality system that is compliant with ISO 17025, for which accurate calibration standards and reference materials are essential elements. Calibration solution CRMs are critical for both instrument calibration and research while matrix CRMs are important to verify the complete analytical procedure, from extraction of shellfish to data analysis. A matrix CRM is very useful for inter-laboratory studies, proficiency testing schemes, and development and validation of new methods. The use of both calibration solutions and matrix CRMs is recommended. The NRC-IMB Certified Reference Material Program (CRMP) is a unique program that produces CRMs for marine toxins. This poster outlines the steps involved in the preparation of both calibration solution and matrix CRMs for lipophilic toxins. The lipophilic toxins include the okadaic acid group, the pectenotoxins, the azaspiracids, the yessotoxins, and the cyclic imine group. The greatest challenges of working with lipophilic toxins include their availability and stability. CRMP has been able to produce these new products with the help of many international collaborators.

PO.08-28
Impacts of toxic cultures of the cyanobacterium *Microcystis aeruginosa* on selected immune parameters of the freshwater zebra mussel, *Dreissena polymorpha*

Session: PO.08 - Toxicology

SC Culloty, Guillaume Juhel, J O'Halloran, RM O'Riordan, J Davenport

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In vivo experiments were conducted with laboratory cultures of *Microcystis aeruginosa*, with different degrees of toxicity, to assess the impacts of microcystins on the immune system of the freshwater zebra mussel, *Dreissena polymorpha*. Mussels were fed three toxic cyanobacterial strains, with different toxic profiles (presence of MC-LR and MC-LF)

and one non-toxic reference strain, over a three-week period. Immunological analyses were carried with mussels sampled at 0, 7, 14 and 21 days.
Results showed an increase in total haemocyte concentration after two weeks and a subsequent decrease after this period. Furthermore, increased strain toxicity appeared to be associated with a greater decrease in cell density. A higher proportion of granulocytes was observed in the very toxic strains after one week of exposure. Phagocytosis results revealed that the presence of MC-LF lowered the ability of the mussels to phagocytose yeast particles and this pattern was even more marked after three weeks of exposure. The concentration of defence enzyme, lysozyme, was variable but increased after two weeks of exposure and decreased subsequently.
In conclusion, microcystins (particularly MC-LF) elicited an immune response after one week of exposure and gradually weakened the bivalve's immune system, demonstrating another sublethal effect of these toxins on bivalves.

PO.10-29
Ecological, morphological, and toxicological analysis of an unusual dinoflagellate, *Amphidinium massartii*

Session: PO.10 - Ecophysiology & Autecology

TJ Cyronak, CR Tomas

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Amphidinium carterae, an important harmful algal species that produces powerful antifungal and hemolytic compounds, amphidinols, and cytotoxic macrolides, amphidinolides, is ubiquitous in coastal waters (Satake *et al*. 1991, Ishibashi and Kobayashi 1997). Samples observed from coral rubble contained an *Amphidinium* sp. with the unusual morphology of completely circular cells. Preliminary genetic analysis by sequencing of LSU and SSU rDNA regions revealed a close relationship between the unknown *Amphidinium* sp. and *A. massartii*. The unusual circular morphology of *A. massartii* has not been reported in previous literature. An examination of this morphology under temperature and nutrient regimes is conducted. Even though it is considered a toxic algae species, little information beyond the description exists for *A. massartii*. *Amphidinium massartii*'s genetic proximity to *A. carterae* is cause for interest in the production of bioactive compounds. Two assays used in the toxin detection of *A. carterae*, a hemolytic assay and antifungal bioassay, offer good measurements of relative toxicity (Echigoya *et al*. 2005). This unusual species of *Amphidinium* is examined for its ecological, morphological, and toxicological properties.

PO.13-50
Ten years of monitoring for toxic species of phytoplankton in the Gulf of Gabes (South-East Tunisia)

Session: PO.13 - Regional events

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Because of the sanitary risks and environmental problems caused by toxic phytoplankton species, surveys and detection of these organisms are now a focus area for scientists and marine exploitation.
In Tunisia a monitoring program was launched in 1995. For the shellfish area in the Gulf of Gabes, we defined 17 sampling stations generally known for eutrophication, and more than 35 stations in open sea.
The toxic phytoplankton along these coasts is limited to 4 dinoflagellate species (*Alexandrium minutum*, *Prorocentrum lima*, *Protoceratium reticulatum* and *Karenia selliformis*), *Karenia selliformis* being the most toxic during the ten years of monitoring. This species is widely distributed along the coasts, especially in the Bougrara Lagoon where we frequently observe red tides.

PO.13-23
Remarkably high level of domoic acid detected in a bivalve *Spondylus versicolor* in Vietnam

Session: PO.13 - Regional events

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Recently, we reported that a significant level of domoic acid (DA), a causative toxin of ASP, occurs in the bivalve *Spondylus* collected from various parts of tropical areas, including Vietnam. DA-producing organism(s) therefore probably occur widely in tropical areas. In the present study, we detected DA around the safety consumption level (1.93 + 1.29 μg/g, n=3) in *Spondylus versicolor* collected from Nha Phu Bay on May 30th, 2005. Thirty specimens were therefore reared in running filtered seawater in an aquarium, and the change of DA level in the specimens was analyzed. During 18 weeks of rearing, no significant decrease of DA level was observed, suggesting that this species maintains accumulated DA for a long period. On September 29th, 2005, specimens of the same species collected from the same area showed remarkably high levels of DA (109.47 + 47.66 μg/g, n=10), which is much higher than the safety consumption level. DA in these specimens was mostly concentrated in the digestive gland. The results suggest that DA of *S. versicolor* originates from microorganism(s) ingested by *S. versicolor*. Probably, the causative organism of DA shows a seasonal bloom pattern. Further surveys of DA in plankton species in tropical waters are required.

PO.03-05
The situation of ciguatera fish poisoning in French Polynesia from 2000 to 2004

Session: PO.03 - Public health

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Ciguatera Fish Poisoning (CFP) is caused by the ingestion of tropical reef fish which have bio-accumulated ciguatoxins in their tissues. This phenomenon is well known by local people particularly in remote archipelagos of French Polynesia where the population still depend on fish resources.
This work presents the situation of

CFP as illustrated by the epidemiological data provided by the Public Health Directory of Tahiti, as well as fish toxicity data obtained in our laboratory from 2000 to 2004. Although probably underestimated, an average of 600 cases of CFP is recorded each year within the five archipelagos composing French Polynesia (i.e. Society, Tuamotu, Gambier, Autrales and Marquesas). In 2004, 42% of the cases occurred in the Tuamotu Archipelago.
Data obtained from epidemiological forms completed by health agents regarding the symptoms experienced by the patients and the fish species implicated in CFP cases are compared between all five archipelagos.
Among the 50 fish species identified, the most often incriminated species belong to longface emperor, leopard coral grouper, humpback red and two-spot snappers, striated surgeonfish and boomerang triggerfish.
Toxicity data obtained via the Receptor Binding Assay for various specimens of fish collected from these five archipelagos are consistent with these data.

PO.10-30

Fibrocapsa japonica: a potentially harmful raphidophyte in Dutch coastal waters

Session: PO.10 - Ecophysiology & autecology

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Ecophysiological studies on a West European strain of *Fibrocapsa japonica* indicate that this raphidophyte must be able to maintain itself in Dutch coastal waters. The experimental data have now been correlated to extensive phytoplankton field data of the Dutch coastal waters over the period 1990-2003. The Dutch coastal zone clearly meets the ecophysiological requirements of *F. japonica*; the area possibly belongs to its northern biogeographic boundary, explaining why vegetative cells have never been observed at higher latitudes. Moreover, the field data strongly suggest that besides *F. japonica* other raphidophytes such as *Chattonella* spp. and *Heterosigma akashiwo* have established themselves in this region. In the surface water samples, *F. japonica* and *Chattonella* spp. have been observed almost the year round, even at low temperatures just above 4 °C. *Fibrocapa japonica* can dominate the phytoplankton community in Dutch coastal waters during short term blooms, not only in summer but also at lower temperatures (< 15 °C) during spring. Despite the presence of haemolytic and toxic components in this raphidophyte, harmful events have not been observed.

PO.05-43

Application of capillary electrophoresis-mass spectrometry to the determination of lipophilic marine toxins

Session: PO.05 - Toxin analysis

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Capillary Electrophoresis is being applied in this work for the analysis of yessotoxins as an alternative to the conventional HPLC method, which is the most commonly used. Coupling of capillary electrophoresis with mass spectrometry (CE-MS) offers a good alternative for sensitive determination of these toxins and also for confirmation of the results obtained.
CE analysis was performed, using 10 mM ammonium acetate at pH 8.5 as running buffer, and mass spectrometric detection were carried out through an electrospray ionisation (ESI) source. An on-line sample preconcentration approach based on field-amplified sample stacking (FASS) was applied to increase the sensitivity. The limit of detection (LOD) achieved was 0.02 μg/mL, which corresponded to 1.25 pg YTX loaded onto the capillary. Under these conditions, Yessotoxin (YTX) and its analogue 45-hydroxy-yessotoxin (45-OHYTX) were clearly determined by CE-MS in several shellfish samples as well as in marine phytoplankton cultures of *Protoceratium reticulatum*. Additionally, the method was successful for determination of other lipophilic toxins present in mussel samples such as pectenotoxin-6 (PTX-6). The results clearly demonstrate the potential of CE, especially when coupled with MS, as an alternative method for determination of these particular compounds.

PO.13-78
Establishment of cultures of HAB organisms from the Mexican Pacific coast

Session: PO.13 - Regional events

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Dinoflagellates are economically important in Mexico as in other tropical countries, as some species are toxic and can produce harmful algal blooms. HAB events are frequently recorded in Mexico. For this reason it is important to culture the producer organisms, which were isolated under a microscope using a micropipette and placed individually into microtiter plates. Twenty-three strains of dinoflagellates causing red tides are maintained in our laboratory. Some have been reported as toxic: *Karlodinium micrum*, *Scrippsiella trochoidea* and *Gymnodinium catenatum*, and others as ichthyotoxic: *Ceratium dens*, *Ceratium fusus* and *Prorocentrum micans*. Clonal cultures are maintained as non-axenic, semicontinuous, L:D 12:12, 18, 20 °C ± 1 °C, illuminations of 166.8 and 90.5 μmol m^{-2} s^{-1}, respectively, using four different culture media. Isolation of HAB producer species are vital for studying the conditions that regulate the growth or control the synthesis of toxic molecules, in order to create models to aid in early detection of red tides events.

PO.12-15
Description of a novel raphidophyte species and genus from Delaware's inland bays, USA

Session: PO.12 - Taxonomy and phylogeny

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Delaware's Inland Bays (DIB), Delaware, USA are subject to numerous mixed blooms of raphidophytes each year. *Heterosigma akashiwo* is a consistently occurring pleomorphic raphidophyte that blooms within a wide range of salinities and temperatures. In the summer of 2004, a unialgal bloom with a density of 200 million cells per liter was detected in low salinity water. Initial analyses via light microscopy led to the misconception that the species was *H. akashiwo*, however much smaller than other field observations in the region. Further molecular analyses of the 18S rDNA sequence indicated that this species is a novel raphidophyte, and phylogenetic analyses place the new species within Raphidophyceae as a new genus. Although this species is morphologically very similar to *H. akashiwo* under light microscopy, transmission electron and confocal microscopy revealed distinct shape and size differences in chloroplasts and some organelles. Although laboratory experiments indicated that this new species grows optimally in low salinity water, pigment analysis indicates the presence of both fucoxanthin and violaxanthin, which are characteristic of marine raphidophytes.

PO.13-08
Ecological analysis of harmful algal blooms for the Bohai Sea area, China

Session: PO.13 - Regional events

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Bohai Sea is a semi-enclosed sea in northeastern China, where harmful algal blooms (HABs) break out frequently and cause large economic losses. The present study analyzed spatial and temporal distribution, causative species of HABs and ecological conditions in the Bohai Sea for the period from 1950 to 2004, and discusses relevant environmental factors. HABs increased in frequency and affected-areas with peaks in 2001 and 1999. The dominant causative species was *Skeletonema costatum* before the 1990s and *Noctiluca scintillans* after 1992. The high blooming season was July-August in the 1980s and 1990s and it shifted to June-August during 2000-2004 for the entire Bohai Sea. Results show Dalian Bay and Bohai Bay to be two high-HAB frequency areas, and differences in high timing and frequency occurrence of HABs are due to local meteorology, nutrient and species. The increase in occurrence frequency and affected-areas of HABs are related to increase in eutrophication, but the improved methodology and improved monitoring enable more HAB to be identified.

PO.13-90
Aquaculture development and potentially harmful microalgae in Senegal

Session: PO.13 – Regional events

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During the last three decades three massive fish kills have taken place in Senegal, however none of them have been associated with the presence of harmful algae. Also, there are no official records of DSP, PSP, NSP or ASP. Shellfish (bivalves) production is mainly estuarine; the mean yield is 2,000 $t.y^{-1}$ locally consumed. But, new oyster and shrimp farm productions have been set up in Saloum Estuary during the last two years. Freshwater fish culture is semi-intensive but new political options are ongoing for an industrial production using more intensive systems. Production is less than $100t.y^{-1}$. Particular attention was paid to cyanobacteria blooms in intensive *Tilapia* production research (high phytoplankton concentration and their use by *Brachionus plicatilis*), but no negative impact or fish mortalities due to cyanotoxins have been observed. The growing focus on exploitation of marine and freshwater shellfish and fish makes it relevant to monitor more detailed for the occurrence of potentially harmful microalgae. In the project ESIT/CSE, receiving satellite images from MERIS and MODIS at 250 and 1,000m resolution, the NODC team and CSE have joined up and follow ocean colour, phytoplankton bloom and eventually red tides. However, small scale monitoring in e.g. existing or planned aquaculture sites is also needed.

PO.05-19
LC/MS-MS determination of paralytic shellfish poisoning (PSP) in seafood by application of a new hydrophilic interaction liquid chromatographic (HILIC) column

Session: PO.05 - Toxin analysis

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Paralytic shellfish poisoning (PSP) is caused by a group of approximately two dozen naturally occurring potent neurotoxins. Consequently, the development of analytical methods for the qualitative and quantitative determination of the toxins associated with PSP is an important task. In particular, the quality control of potentially contaminated seafood requires exact quantification of the PSP toxins with regard to international regulations for public health protection and international commerce. The broad toxicity range of different PSP toxins has challenged analytical chemists to develop accurate and reliable analytical methods.
Chemical methods for PSP determination are based on chromatographic separation and derivatization of the toxins prior to fluorescence detection. However, these HPLC methods complicate the on-line coupling to a mass spectrometer (MS). The new developed LC/MS-MS method is based on chromatographic separation of the PSP toxins using a zwitterionic-HILIC column. This stationary phase allows complete separation of all relevant PSP toxins in seafood. Low concentration of volatile buffer substance and absence of ion-pair reagents increase the sensitivity of the MS detection.

Literature:

[1] B. Luckas, L. M. Botana (ed.), Seafood and Freshwater Toxins

2000, 173.

PO.15-17
The monitoring programme for harmful algal blooms in shellfish production areas in Catalonia. Long term data and impact on aquaculture

Session: PO.15 - Monitoring

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The monitoring programme for HABs in shellfish production areas in Catalonia focuses mainly on shellfish harvesting areas in sandy sediments along the shore (mainly natural production sites of clams) and production areas within the semi-confined areas (mainly oyster and mussel cultures). HAB incidences in production areas include recurrent DSP (e.g., *Dinophysis sacculus*), PSP (e.g., *Alexandrium minutum*) and to a much lesser extent ichthyotoxic events (*Karlodinium* spp.). An estimate of the percentage of closure periods indicates that annually these figures have increased since 1989, DSP events being the major cause for administrative closures. For the period 2002-2005 closure time in shellfish production areas reached maxima of 23 % (84 days in Alfacs Bay during 2002 for DSP) and 17% (62 days in Alfacs Bay during 2005 for DSP). The economic impact of closures is irregular and depends on the time of the year closures occur. This is due to unbalanced commercial demand along the year but also to the threat that increased temperatures in summer represent to unharvested production in semi-confined areas. This can lead to massive mortality of mussels and oysters when closures occur just before harvesting periods.

PO.06-21
Water mass differentiation using PARAFAC modeling of EEM fluorescence

Session: PO.06 - Population dynamics

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The optical properties of coastal waters, as excitation-emission matrix (EEM) fluorescence (220-455nm excitation, 249-700nm emission) and CDOM absorption, were used to map a complex coastal environment off the west coast of Florida (U.S.A.) which receives multiple estuarine inputs and records nearly annual occurrences of *Karenia brevis* blooms. Sampling was conducted during the initiation and continuation of an extended and extensive bloom. Samples represented a wide range of CDOM absorption along an estuarine-coastal gradient and EEM data were corrected for inner filter effects, blue-shifting the raw fluorescence data. EEM data were subsequently normalized to prevent undue weight being assigned to high CDOM samples, fluorescent components were identified with the linear un-mixing technique of parallel factor (PARAFAC) analysis, and components confirmed with split-half analyses to identify two dominant multi-modal components to coastal fluorescence with both a direct and an inverse relationship with salinity. Other spectral regions of high variation were identified in the pool of samples and compared with fluorescence peaks in the literature. Although factors did not correlate with cell count or presence of *K. brevis*, spatial patterns of optical properties and salinity were used to identify the origins of coastal water masses and potential sources of entrained nutrients.

PO.10-38
Pseudo-nitzschia along the south-central coast of Vietnam: abundance, distribution, T-S characteristics, and growth rate of cultures and natural populations

Session: PO.10 - Ecophysiology & autecology

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Pseudo-nitzschia species are widely distributed along the coast of Vietnam with abundance distribution varying in time and space. Along the southern-central coast, however, they tend to occur in high abundance during the rainy season, when nutrient loading from land/rivers is high. The seasonal variation shows local differences depending on the hydro-chemical characteristics of each embayment along the coast. The *Pseudo-nitzschia* assemblages were observed as high as 400.000 cells/L, which is close to the level of shellfish harvesting closure in Europe. Temperature and salinity compared with cell abundance indicated that *Pseudo-nitzschia* may adapt to a wide range of temperature and salinities. In Nha Phu Lagoon there were at least three different *Pseudo-nitzschia* assemblages that grew at (1) low temperature and low salinity; (2) high temperature and low salinity; and (3) high temperature and high salinity, while they were stenohaline but eurytherm in Nha Trang Bay and the northern coast of Binh Thuan waters. *Pseudo-nitzschia cuspidata* isolated from Nha Trang Bay showed the highest growth rate at 35 PSU and 30 °C (1.8 d^{-1}), but grew well at a range from 20-30 °C and 25-35 PSU. This species may be a representative of the high temperature and high salinity populations in Nha Trang Bay.

PO.13-84
Toxicity of *Pseudo-nitzschia* spp. in estuarine and shelf waters of Louisiana, USA

Session: PO.13 - Regional events

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Pseudo-nitzschia spp. are frequently widespread and highly abundant in Louisiana coastal waters. Seven species have been identified, including, *P. pseudodelicatissima*, *P. delicatissima*, *P. multiseries, P. pungens*, *P. multistriata*, *P. subfradulenta*, and *P. brasiliana*. Although several of these are known producers of domoic acid (DA), no human illness has been reported. In order to determine if DA was being produced and, if possible, by which species and under what environmental conditions, DA content was measured in net tow samples (35

micron) from 1994-1998, using a receptor-binding assay. Samples were selected because they had a high *Pseudo-nitzschia* abundance, and species composition was determined by SEM on many samples. Most (80% of 106) samples contained DA, with concentrations ranging from 0.01 to 28.65 pg/cell. Preliminary analysis of the data suggests that salinity and dissolved inorganic nitrogen was higher when DA was present and there was a significantly higher percentage of *P. multiseries* and *P. delicatissima* present when DA was present. *Pseudo-nitzschia* spp. present in Louisiana coastal waters do produce DA and, thus, represent a threat to human and ecosystem health.

PO.10-28

Delayed fluorescence as an indicator of nutrient limitation in *Prorocentrum minimum*

Session: PO.10 - Ecophysiology & autecology

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Primary productivity and growth of algae is frequently limited by the availability of nutrients, especially nitrogen and phosphorus. The ability to identify factors limiting algal growth is of considerable importance for marine ecology.
In our study, delayed fluorescence (DF) was used to detect changes in photosynthesis due to nitrogen and phosphorus limitation in laboratory cultures of *Prorocentrum minimum*. DF is a long-lived light emission from photosynthetic organisms after illumination. DF decay curve reflects the state of the photosynthetic apparatus.
Monitoring of DF decay curves of light-adapted and dark-adapted samples, DF intensity versus irradiance response, chlorophyll content, cell concentration and maximum quantum yield of PSII (Fv/Fm) was performed for 4 days on cultures growing in nitrogen- and phosphorus-limited media.
Significant changes in DF kinetics with regard to medium composition were observed. Chlorophyll content and Fv/Fm were reduced compared to the control for cultures in nitrogen-deprived media. The changes in DF intensity versus irradiance response showed differences in the absorption of light.

PO.08-19

Uptake and elmination of DST in mussels, oysters and scallops

Session: PO.08 - Toxicology

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Mussels (*Mytilus edulis*), oysters (*Ostrea edulis*) and scallops (*Pecten maximus*) were exposed to natural occurrences of *Dinophysis* spp. Samples were taken for six weeks during toxin accumulation followed by two months of elimination in toxin-free waters. Large species differences in accumulation were seen, with mussels accumulating 10 to 20-fold higher toxin concentrations compared to oysters and scallops.

Due to sudden mortality after the accumulation period we could not evaluate toxin elimination in the scallops. The oysters, having accumulated less DST than the mussels, also showed faster toxin elimination rates. DST concentration in the oysters did not exceed half the regulatory limit, despite high water column toxicity that resulted in concentrations almost 10 times the regulatory limit in the mussels.

PO.05-18
Deoxy cylindrospermopsin, detection in the benthic freshwater cyanobacterium *Lyngbya wollei* from Australian streams

Session: PO.05 - Toxin analysis

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Cylindrospermopsin and its deoxy analogue have been detected in a number of cyanobacteria throughout the world. Previously in Australia these toxins have been reported in *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum*. In both these species the deoxy-cylindrospermopsin levels are less than 10 % of the predominant toxin, cylindrospermopsin. *Lyngbya wollei* specimens from several streams in South East Queensland were found to contain deoxy-cylindrospermopsin as the predominant toxin with only low levels of cylindrospermopsin. The level of toxin in the water was below the detection level of 0.2 micrograms per Litre in contrast to the other two species mentioned which often give toxin levels in the surrounding water at similar concentrations to the toxin content of the cells. This is the first report of these toxins being detected in a benthic cyanobacterial species.

PO.12-07
Haplo-diploid life cycles in the genus *Chrysochromulina* (Haptophyta)

Session: PO.12 - Taxonomy and Phylogeny

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A heteromorphic haplo-diploid life cycle appears to be common among haptophytes and has been found in *Chrysochromulina polylepis*. To assess whether other *Chrysochromulina* species may have a haplo-diploid life cycle, coding and non-coding ribosomal DNA regions, body scale morphology, cell size and ploidy level were examined in 30 strains representing 16 *Chrysochromulina* species. All described species had distinct SSU rDNA sequences, except strains of *C. hirta* and *C. ericina*, which were identical in this region, suggesting that they have diverged too recently to have evolved differences in this gene, or alternatively are conspecific. Two ploidy levels differing by a factor of two assumed to represent haploid and diploid stages, each capable of vegetative cell divisions were found in five species: *C. ericina, C. hirta*, *C. kappa*, *C. rotalis* and *C. polylepis*. Haploid and diploid cells of *C. kappa* and *C. ericina* had similar scale morphology, but were heteromorphic in *C. hirta*, *C. rotalis* and *C. polylepis*. All strains within a species had identical ITS1 rDNA sequences. These results suggest

that other *Chrysochromulina* species in addition to *C. polylepis* have a haplo-diploid life cycle with an alternation between heteromorphic or isomorphic motile stages.

PO.12-14
Morphology and ultrastructure of *Chattonella* aff. *verruculosa* (Heterokontophyta)

Session: PO.12 - Taxonomy and Phylogeny

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Since 1998 the flagellate *Chattonella* aff. *verruculosa* (Dictyochophyceae), has formed recurrent blooms in the North Sea and Skagerrak causing fish mortalities. Cells were isolated from the 2001 bloom off the south coast of Norway and strains established and compared with the *Chattonella verruculosa* Hara et Chihara type strain NIES 670 from Japan. The cells in the Norwegian isolates were very variable in size and form, being large oblong (= 40 µm long) to small rounded (<10 µm in diameter) with two unequal heterokont flagella. The chloroplasts are numerous (up to 30-35) and yellow brown. The cells are covered in oval mucocyst-like bodies. The nucleus is located centrally in the anterior part of the cell close to the basal bodies, and the Golgi body is situated on the lateral side of the nucleus. The flagellar transition region contains two proximal rings (two gyre helix) and distal rings (helix), a combination of features found only within the Dictyochophyceae. Fibrous roots connecting the basal bodies and microtubular roots, consisting of a few microtubules, are present, but have not been studied in detail. Strain NIES 670 has a morphology and ultrastructure similar to that of *Chattonella* aff. *verruculosa* from Norway, but they differ genetically.

PO.01-15
Molecular tools for the identification of *Pseudo-nitzschia* in Catalan waters, Spain

Session: PO.01 - Genetics

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A new project focusing on the development of molecular tools for the identification of toxic phytoplankton in Catalan waters has been initiated. This project arises from the need to characterise both in culture and in the field harmful microalgal species and to develop specific molecular tools for their identification. For this purpose, characterisation in terms of morphology, growth and rRNA sequences of *Pseudo-nitzschia* spp. cultures isolated from the NW Mediterranean coast is currently underway. Here, we report the initial results obtained on eight clonal cultures of *Pseudo-nitzschia* spp. isolated from Alfacs and Fangar Bay in the Ebro Delta during the winter 2006. Based on scanning electron microscopy, these isolates were identified as *P. delicatissima* and *P. calliantha*. Morphological description of *P. delicatissima* isolated from both Alfacs and Fangar bays and *P. calliantha* isolated from Alfacs Bay are presented in this study. The identity of the species was confirmed by analysis of the ITS1, 5.8S and ITS2 of the nuclear-encoded rRNA sequences. Intraspecific and interspecific variability of the cultures was also assessed by analysis of the nuclear-encoded rRNA in order to develop species-specific oligonucleotide probes targeting the rRNA for the identification of *Pseudo-nitzschia* spp. by fluorescent *in situ* hybridisation (FISH) techniques.

PO.06-06
Seasonal dynamics of a *Planktothrix rubescens*-dominated phytoplankton community and toxic compounds in Lake Albano (Rome, Italy)

Session: PO.06 - Population dynamics

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Lake Albano is situated in the Colli Albani Volcano 25 km southeast of the city of Rome. Physico-chemical measurements and analyses of water were made at discrete depth intervals across the full depth profile of the lake (0-160m). Analyses of phytoplankton toxins were made on net samples using MALDI-TOF mass spectrometry and observations and counts made on samples from discrete depths between 0-30m using a 1-L Niskin bottle. Sampling was made on five occasions between 2005 and 2006 to incorporate complete seasonal variation. The lake is classified meso-eutrophic; based on average epilimnion total phosphorus (5-92 µg/L), total nitrogen (365-809 µg/L) and chlorophyll a (3-14 µg/L). The phytoplankton community was variably dominated by the cyanobacterium *Planktothrix* (*Oscillatoria*) *rubescens* with a distribution across a small depth range under thermal stratification. With an increase in mixing depth in May-05 down to 100m and Jan/Mar-06 full overturn, *P. rubescens* was distributed throughout the epilimnion and caused surface blooms. Throughout the study period there were three variants of microcystin identified, the major variant, present in every sample, was [D-Asp]-MCYST-RR. The variants, (Asp)-MCYST-LR and MCYST-YR where absent in autumn. This is the first study to show the diversity of microcystins produced by *P. rubescens* in Lake Albano.

PO.13-18
Distribution of toxic *Dinophysis* species and contamination of shellfish along the Doukkala coast (Moroccan Atlantic water)

Session: PO.13 - Regional events

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The distribution of *Dinophysis* species and the contamination of shellfish in the Doukkala coast (Moroccan Atlantic water) were studied.
During the monitoring program, samples were taken bimonthly from 2004 to 2005 at about eleven sampling stations, situated on 2 lagoons and open sea sites along the coast.
The presence of toxic species of *Dinophysis* varied seasonally, with maximum abundance registered in

summer. Among the *Dinophysis* cells, some individuals were morphologically similar to *D. acuminata*, *D. sacculus*, *D. acuta* and *D. caudata*. At the beginning of August 2004, *Dinophysis* spp. were found at Sidi Moussa Lagoon (Atlantic water) with a high density (1.3 10^3 cells/l), but DSP was not detected on the analysed clams. However, in open sea areas (Jemaa ouled Ghanem and Cap Beddouza) high levels of DSP were found in *Mytilus galloprovincialis* during the summer of 2005, although *Dinophysis* spp. was present at lower density. During the monitoring program, the spatial distribution of *Dinophysis* spp. was not homogeneous, and contamination by DSP can occur with very low density of *Dinophysis*, causing the closing of production zones.

PO.06-09

Follow up of an autumn bloom of *Dinophysis acuta* in NW Iberia: along-shore transport versus in situ growth.

Session: PO.06 - Population dynamics

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Previous observations on mesoscale dynamics of *Dinophysis acuta* in NW Iberian waters suggest that Aveiro functions as the 'epicentre' for initiation (May-June) of *D. acuta* blooms. A northwards shift of the cell maximum during autumn blooms was interpreted as a predominance of physical advection of cells and/or conditions favouring dinoflagellates growth during the transition from upwelling to winter conditions. Record concentrations of *D. acuta* (140,10^3 cells/L) were observed off Aveiro between August and November 2005. In Galicia, September-mid October estimates indicated good *in situ* growth (μmin = 0.22 d^{-1}), low cell densities (<10^2 cells/L), and even negative net growth (estimated from weekly monitoring of 18 stations in Vigo and Pontevedra) of *D. acuta*. In contrast, record autumn concentrations of *D. acuta* (> 17 · 10^3 cells/L in integrated hose-samples) were observed in early November together with negligible division rates (< 0.1 d^{-1}) and high (up to 0.60 d^{-1}) net growth. Analyses of spatio-temporal variability in cell numbers, in situ division rates, SST images, current-velocity measurements and physical-model simulations support the view that in early November 2005, the shift of *Dinophysis* maxima from Portugal to Galicia was the result of a northwards physical transport.

PO.07-13

A fuzzy logic model for *Alexandrium minutum* proliferations in harbours of the Catalan coast (NW Mediterranean)

Session: PO.07 - Ecology and oceanography

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The dinoflagellate *Alexandrium minutum*, a PSP producer, forms recurrent blooms in many estuaries and semi-enclosed marine areas. We explored the feasibility of designing a fuzzy logic model based on historical ecological information and routinely monitored meteorological variables, to predict the occurrence and intensity of *A. minutum* proliferations in two selected harbours ('Olímpic' Harbour of Barcelona and Arenys Harbour) of the Catalan coast (NW Mediterranean). Fuzzy logic models represent the knowledge in terms of IF-THEN rules and allow the combination of partial truth values with a certain uncertainty within the available data set. The model was developed for the Olímpic Harbour and assumed that bloom timing and intensity depended on the coincidence of more or less favourable seasonal windows with a two to four-week spell of favourable weather conditions including lack of heavy precipitation and of strong winds. The model tended to exaggerate the foreseen bloom intensity, but successfully predicted the 6 high biomass blooms (1 in the Olímpic and 5 in Arenys) recorded in the studied harbours between 2000 and 2002. This result suggests that, although based on limited understanding of the processes affecting bloom dynamics, the developed fuzzy model could be helpful in providing insight for management measures.

PO.03-01

2005 New England paralytic shellfish poisoning (non)-event: risk management success story

Session: PO.03 - Public health

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The largest recorded *Alexandrium fundyense* bloom since 1972 occurred off the New England coast from May to July 2005. This resulted in paralytic shellfish poisoning (PSP) toxins greatly exceeding the action level of 80 micrograms saxitoxin equivalents per 100 grams tissue. At the request of the US Food and Drug Administration (FDA), the US National Marine Fisheries Service (NMFS) closed approximately 15,000 square miles of federal waters in the northwestern Atlantic Ocean on 14 June. Since the closure shellfish toxicities have been monitored using the receptor binding and/or mouse bioassays. Toxicities varied with species, with maximum levels of 2045, 4378, and 4200 micrograms per 100 grams reported in whole scallops from federal waters and in blue mussels from waters off Massachusetts and Maine, respectively. Toxicity decreased over time with depuration rates differing between species. Analytical data supported reopening part of the closure on 9 September (except for whole and roe-on scallops); the northern area

has remained closed due to insufficient sampling. Due to timely and effective state responses and the assistance given by FDA and NMFS, there were no human PSP illnesses despite remarkably high toxicity in the unmarketed product.

PO.06-02
Dinoflagellate diversity and abundance in seven Belizean coral reef-mangrove lagoons: a test of Margalef's Mandala

Session: PO.06 - Population dynamics

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Toxic and non-toxic dinoflagellates are abundant in the waters of the Belizean Central Lagoon, Atlantic Barrier Coral Reef Mangrove Ecosystem. Dinoflagellates are costal and off shore planktonic, and benthic species. Most often bloom forming dinoflagellate observed are: *Bysmatrum caponii*, *Dinophysis caudata*, *Gonyaulax grindleyi*, *Peridinium quinquecorne*, *Gonyaulax polygramma*, *Gonyaulax spinifera*, *Lingulodinium polyedrum* and *Pyrodinium bahamense* var. *bahamense*. Nineteen bloom-forming species, and half of those identified are known toxins producing species. Network of coral ridges of semi-enclosed ponds of typological diversity and complex ecology limit water exchange from the lagoon. The unusual hydrography of the mangrove embayments, and nutrient input from natural sources enhance development of blooms. Bloom of *G. polygramma* may reach cell densities of 1-3 x 10^6 l^{-1}. Dissolved ammonium is the naturally produced nutrient at 0.1-6.0 µmol L^{-1} maintains the blooms of dinoflagellates. The above conditions influence the ability of cells to concentrate and proliferate into species-specific blooms that favor dominance toward dinoflagellate species. Margalef Mandela predicted that marine environments with low turbulence and high nutrient inputs would favor dinoflagellates. As a consequence this species shift may cause altered food web dynamics and the prevalence of toxins in the microbial food chain.

PO.07-17
Advection, stratification and harmful algal bloom development in the southern Benguela upwelling system

Session: PO.07 - Ecology and oceanography

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The west coast of South Africa experiences problems associated with harmful algal blooms (HABs), which are typically attributed to dinoflagellate species. Wind-driven upwelling, which is a feature of the southern Benguela, plays a critical role in the algal dynamics of the region. Physical processes important to bloom situations are mixing and stratification, which affect species selection; and advection, which affects bloom transport, retention and shoreline impact. Data from a multi-sensor mooring, providing HAB focussed real-time and time series data, has enabled the detection of high biomass blooms and allows further insight into the roles of stratification and advection in the development of such events. Dominant periods in wind, current and temperature data are found at inertial/ diurnal frequencies and around 3 and 9 days, demonstrating the link between wind and current reversals, and changes in temperature structure. Currents lag wind reversals by around 12 hours, with relaxation of upwelling winds leading to poleward advection of warm water creating stratification and conditions conducive to bloom formation. On a short time scale inertial oscillations create semi-retentive circulation patterns, and enhance mixing through the introduction of vertical shear into the water column. Mooring data will be used to illustrate these processes.

PO.07-18
Going beyond nutrients: role of environmental factors in shaping harmful algal blooms in estuarine waters

Session: PO.07 - Ecology and oceanography

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Nutrient availability is often considered a major contributory factor in algal productivity. However, other environmental components can selectively displace individual species even under eutrophic conditions. Therefore, it is necessary to identify other contributory factors that promote HABs if we are to develop better predictive models for bloom occurrence and duration. This study focused on four potentially harmful algae (dinoflagellates *Gyrodinium instriatum*, *Karlodinium micrum*, and *Prorocentrum minimum*, and cyanobacteria *Cylindrospermopsis raciborskii*) that occur in the estuaries of North Carolina, USA. Multivariate analyses were conducted on data collected between 2000 and 2005 in three tributaries (Neuse, New, and Pamlico Rivers) of the Albemarle-Pamlico estuarine system. In general, *G. instriatum*, *K. micrum*, and *C. raciborskii* were intolerant to high flow conditions, as abundances greater than 1,000 units/ml only occurred at flows less than 50 m^3 s^{-1}. Whereas*,* *P. minimum* abundance was as high as 21,000 units/mL at flows greater than 230 m^3 s^{-1}. *Prorocentrum minimum* populations were also more pronounced during cooler temperatures, higher NOx-levels, and lower salinities. Both *C. raciborskii* and *G. instriatum* were more prevalent during periods of lower salinities, and *K. micrum* was most abundant during warmer temperatures, higher salinities, and higher total phytoplankton abundances.

PO.07-16
The Ebro Delta coastal embayments, a GEOHAB pilot site for the study of HAB population dynamics

Session: PO.07 - Ecology and oceanography

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Coastal embayments of the Ebro Delta in the NW Mediterranean include Fangar and Alfacs Bays. Both bays are active sites for shellfish production of mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*) and have been subject over the years to extensive studies in relation to HABs. We present herein the first year results of a collaborative research project between NRC (Canada), CSIC and IRTA (Spain) concerning HAB population dynamics in Alfacs Bay. This project has recently started (2005) and in conjunction with the local monitoring program, aims to give knowledge on the occurrence of HAB events using Alfacs Bay as a pilot site for coastal embayments. For this purpose marine toxins in bivalves and phytoplankton were recorded together with environmental and physical parameters that could be crucial for the understanding of HABs dynamics. Evaluation of toxicity over a 52-week period shows DSP episodes with co-occurrence of YTX in shellfish in Alfacs Bay. Complementary results include the distribution of harmful species, the description of toxins in shellfish and phytoplankton through analytical chromatography, the cytotoxicity of phytoplankton recovered from net hawls, chlorophyll-a quantification, seston estimation through light absorbance evaluation and description of environmental and physical parameters.

PO.10-33

First study of *Gymnodinium catenatum* sexuality in natural samples from Galicia´s coasts (NW Spain)

Session: PO.10 - Ecophysiology & autecology

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Gymnodinium catenatum Graham is an unarmoured, cyst-forming dinoflagellate species responsible for outbreaks of paralytic shellfish poisoning (PSP). During November and December of 2005, *G. catenatum* bloomed in the Galician coasts (NW coast of Spain). Vegetative cells, fusing gametes, planozygotes and resting cysts were counted in fixed samples which morphologies and sizes were previously compared for calibration to the observed in living ones. The maximum values recorded were of 86.000 vegetative cells/L, 1000 fusing gamete pairs/L, 18,000 planozygotes/L and 480 cysts/L. Living sexual stages were isolated to check behaviour and viability. Apart from the previously cited sexual stages, other unreported behaviours of *G. catenatum* sexuality in natural samples, such us planozygote division, bilobulated cysts and chains of resting cysts were documented, photographed and compared to laboratory studies. Sediment samples were taken 3 months after the bloom. Up to 671cysts/cc were counted, and after isolation, high values of germinability and viability were obtained.

PO.13-80

Fish and wildlife mortalities associated with the 2005 Florida red tide

Session: PO.13 - Regional events

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Throughout 2005, the Florida Gulf coast experienced one of the most severe *Karenia brevis* red tide events in recent decades with significant impacts on aquatic animals and benthic systems. The earliest mortalities began with offshore fish kills in January. In early March, mortalities of the Florida manatee increased, followed in the summer by wide-scale strandings of bottlenose dolphin and multiple species of sea turtles. By August, a large-scale bloom-related hypoxic/anoxic zone developed offshore of southwest Florida, affecting hundreds of square miles of patch reefs and other benthic communities. Divers reported widespread benthic mortalities, including corals, multiple invertebrate and vertebrate taxa and multiple reef fish species. Large numbers of aquatic birds were affected throughout the bloom period.
The temporal sequence of the mortalities reflects movement of the bloom over time as well as the ecology of the affected animals and routes of toxin exposure. Analyses demonstrated the accumulation of brevetoxins in the food web, with exposure confirmed in multiple animal species. With the exception of the fish kills and benthic mortalities associated with the hypoxic zone, ingestion is believed to be the primary lethal route of exposure in these events.

PO.05-15
First report of the production of spirolides by *Alexandrium peruvianum* (Dinophyceae) from the Mediterranean Sea

Session: PO.05 - Toxin analysis

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Four strains of *Alexandrium peruvianum* obtained from resting cysts in the western Mediterranean in 2002 and 2004 were analyzed for the presence of toxins. No PSP toxins were detected by LC-FD after postcolumn derivatization. Nevertheless, spirolides were detected by LC-MS in the four strains. The major components were 13-desmethyl spirolide C (m/z 692,5) with 90% of the total toxin content, followed by spirolide B (m/z 694,5) (6%) and small quantities of spirolide D (m/z 708,5) (2,7%) and 13-desmethyl spirolide D (m/z 694,5) (2%). Also traces of spirolide C (m/z 706,5) were found. This is the first report of the production of spirolides by this species. Given the great morphological similarity between *A. peruvianum* and *A. ostenfeldii*, in which the main difference is the shape of the sulcal anterior plate, triangular in *A. peruvianum* and rectangular with a left extension in *A. ostenfeldii*, the presence of these peculiar toxins in both species could be an indication of a possible synonymy.

PO.15-29
Development of real-time PCR assays for the detection of *Cylindrospermopsis raciborskii*

Session: PO.15 - Monitoring

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Blooms caused by toxic blue-green algae have repeatedly produced episodes of wild and domestic animal illness and death. Since 2002 the toxin producing *Cylindrospermopsis raciborskii* is frequently abundant from summer to fall in the Caernarvon/Breton Sound Estuary in Louisiana, posing a potential risk of a bloom of this species in that area. Recently (10/04) the species was detected in abundant biomasses in the whole Atchafalaya Basin. Monitoring these areas for the presence of *Cylindrospermopsis* is essential in assessing the potential for bloom formation. However, detection and quantification of *Cylindrospermopsis* is sometimes problematic, especially if individual chains lack the characteristic terminal heterocyst.
To circumvent this problem we developed a real-time PCR assay for rapid detection of the species. In this assay, detection of amplified target DNA requires annealing of fluorescent-labelled probes, resulting in added level of specificity compared with assays based on traditional PCR methodology. This sensitive technique detects even low densities of this species instantly, allowing the creation of real-time species abundance distribution maps, enabling managers to respond immediately to increases in biomass of this harmful species and for timely notification of possible health risks to the public

PO.13-57
Summer *Alexandrium catenella* bloom and the impact on fish farming, in the XI region, Chile

Session: PO.13 - Regional events

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During the summer of 2005 and 2006 a bloom of *Alexandrium catenella* was observed in the northern inland sea of the XI region. This species is well known as a PSP producer. Unusual behaviour of caged salmon and high *A. catenella* abundance, more than 356 cells/mL in surface waters at the fish farm, indicated damage to the fish gill and caused a serious salmon kill at the site.
The cells were distributed mainly in the upper surface layer, numbers diminishing with depth. At one site located in the Guaitecas Archipelago (ca. 44º lat. South) the maximum concentration was 961 cells/mL, representing 86 % of the total phytoplankton community. The bloom began in December 2005, and the maximum abundance of *A. catenella* was observed during the 2nd and 3rd week of January. At all sites with positive presence of the dinoflagellate the sea temperature was higher than 12.0 ºC.

PO.13-55
TTR, a new project of the WESTPAC-HAB programme

Session: PO.13 - Regional events

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IOC/WESTPAC, IOC Sub-Commission for the Western Pacific, has implemented its HAB Programme since 1989, to study biological, ecological and biochemical characters of harmful microalgae and the effects caused by the algae. The programme has recognized the importance of training activities and the IOC/WESTPAC has conducted relevant training courses seven times during 1995-2003, focusing on the development of skills to detect toxic dinoflagellates and toxins in contaminated shellfish. The courses have been funded by the Japanese Government. Significant achievements have ben obtained during these courses. However the obtained skills were sometimes abandoned because the trainees did not have the possibility to apply them in their positions at their home countries. We resolved that the training courses should be upgraded to the 'Training through Research (TTR)' project. This reformation was supported at the Fifth WESTPAC Session held in Nha Trang, Vietnam, in May 2005. Whereas the previous training courses did not include research activities, the TTR programme can be regarded as an international cooperative research programme. Several research groups (RGs) on different scientific subjects were established; the first RG on Cyst mapping, the second RG on HAB species characterization, and the third RG on use of ELISA kit for PSP toxin detection.

PO.03-07
Italian observatory on water and health

Session: PO.03 - Public health

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Italy has enforced the European directives on bathing and drinking waters, and the quality is generally good. Yet, the national organisation in this area suffers from shortcomings in: adequate monitoring programs; lack of integration of institutional actors (at national and local levels); analytical data not always being produced in a quality system; no integrated approach for the surveillance of waterborne diseases; problems of management of data at the national level (informative system).
To face some of these problems, a national Observatory on water and health has been created.
Several Italian surface waters used for drinking and bathing activities are infected by toxic cyanobacteria belonging to the genera *Cylindrospermopsis*, *Plankthotrix* and *Microcystis*. Most recently, in the marine waters blooms of toxic algae such as *Ostreopsis ovata* have occurred. Such blooms have been associated with minor human health effects following exposure to the toxic aerosols from the algae. The Observatory activities will include both issues of freshwater cyanobacteria and toxic marine algae. The informative system will collect and elaborate data on the aspects related to human health,

will provide guidelines for monitoring activities and will propose training courses.

PO.16-16
Spatial and temporal distribution of dinoflagellate cysts in Malampaya Sound, Palawan, Philippines

Session: PO.16 – Life cycles

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Dinoflagellate cyst mapping from the sediment surface were conducted at 26 sampling stations in two basins of Malampaya Sounds, Palawan, from late 1998 to mid-2002. The study focused on the spatio-temporal distribution of cysts of the PSP-producing species, *Pyrodinium bahamense* var. *compressum* (*Polysphaeridium zoharyii* in paleontological nomenclature) in the sediments, to assess the existence of possible 'seed beds', that may serve as source of motile cells. Approximately 22-32 dinoflagellate cyst types were recorded, representing nine motile cell-defined genera and one cyst-defined genus. The cyst assemblages found during all monsoonal seasons were characterized by higher proportions of autotrophic species, which were widely distributed in the surface sediments. A higher density of *Polysphaeridium zoharyii* cysts, which occurred at several stations along the northwest side of the Outer Sound, were observed mostly in deep, small embayments with restricted inlets and muddy sediments. The consistent appearance in time and space of very abundant *P. zoharyii* cysts in the surface sediments of the Outer Sound indicates potential 'seed beds' for blooms of motile cells of *P. bahamense*.

PO.06-05
First record of a large-scale bloom of *Thalassiosira curviseriata* Takano in the East China Sea

Session: PO.06 - Population dynamics

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In the East China Sea (ECS), a large- scale bloom of *Prorocentrum donghaiense* Lu from April to June has been a recurrent phenomenon in recent years. However, the *Prorocentrum* bloom was rather small during the spring of 2005. Instead, there was a large-scale bloom of diatoms from late March to mid-April and from late May to Mid-June. The dominant species was *Thalassiosira curviseriata* Takano, together with *Skeletonema costatum* (Greville) Cleve and *Chaetoceros debilis* Cleve. The cell concentration reached 10^6 cells/L and the bloom area extended for several thousand km^2. *Thalassiosira curviseriata* is a newly recorded diatom species from China and blooms have never been reported before. It mainly occurred in the surface and subsurface waters and the main distribution region in the ECS was at 122°4'-122°13'E and 27°42'-28°59'N. The highest cell

concentration observed was 1.27×10^6 cells/L. *Thalassiosira curviseriata* is a spiral chain-forming diatom with a cell size of 8.3-12.6um. Its characteristics were studied by LM and EM and results are given in this paper.
This work was supported by China 973 program (2001CB409701) and NSFC (40476055).

PO.13-75
A review of harmful algal blooms along the Mexican Pacific coast (1878-2006)

Session: PO.13 - Regional events

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An increase of harmful algal blooms has occurred worldwide over the last few decades. This increase has also been observed in Mexico. The present study aims at updating the information on HABs along the Mexican Pacific. A bibliographical review (60 references) and study of unpublished data indicate 167 blooms, mainly dinoflagellates and diatoms. Of these blooms, 33 took place on the west coast of the Baja California Peninsula; 108 in the Gulf of California; 21 in the tropical Pacific, and 5 in the Gulf of Tehuantepec. The highest number of blooms occurred in Bahía de Mazatlán and Bahía de La Paz. The most commonly reported species (> 30) was the ciliate *Myrionecta rubra*, which is distributed from Punta San Hipólito (27°N, 114°W) to Oaxaca (15°40'N, 96°30'W), followed by *Gymnodinium catenatum*, with more than 25 blooms from the Gulf of California to Oaxaca. The toxicity of *G. catenatum* has been determined in strains from the Gulf of California. Recently, *Rhizosolenia debyana*, *Eucampia zodicacus*, *Chaetoceros debilis*, *Cochlodinium polykrikoides*, *Prorocentrum rathymum*, *P. mexicanum*, *Gyrodinium instriatum* and *Chattonella marina* have been reported to form blooms along the coasts. This review highlights the importance of monitoring programs to understand causes, incidences, toxicity, and effects on ecosystems and human health.

PO.16-12
The bottom cell clusters: a new hypothesis for bloom initiation of cyst-forming dinoflagellates

Session: PO.16 – Life cycles

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Alexandrium catenella causes recurrent toxic blooms (> 1 million cells/l) in the Thau Lagoon (French Mediterranean). The bloom development occurs only in the Angle Creek, which is a small, shallow and semi-enclosed embayment. We mapped the resting cyst distribution, which revealed a low average abundance of cysts in the sediments (40 cysts/ml) and a few accumulation patches (up to 200 cysts/ml). Our experimental investigation of cyst biology revealed synchronous germinations involvong up to 90 % in 4 days. However, the capacity of germling cells to divide and form a new population was very low. These results suggest a very low seeding potential in the creek. In parallel,

biological and hydrodynamical numerical models are being developed for *A. catenella* bloom simulation in the area. In order to reconcile our observations with numerical simulations, it appears that the low inoculum resulting from cyst bank germination must remain concentrated close to the bottom as a condition for successful bloom initiation. Less than 4 days of water column stability associated with a frontal structure reduced the dispersion and supported the cohesion of the bottom cell clusters, allowing the seeding population to reach a critical density for blooming.

PO.01-27
Population genetic structure of *Skeletonema marinoi* - a model species for phytoplankton bloom dynamics

Session: PO.01 - Genetics

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Phytoplankton blooms fuel coastal food webs but can sometimes be harmful due to overwhelming concentrations, production of toxins, or mechanical injury. The genetic diversity of a population is important in determining the response to changing environmental conditions. This diversity may also be important for bloom formation. We have examined the genetic diversity of *Skeletonema marinoi* clones isolated from Gullmar Fjord on the Swedish West Coast. This is a very common species in the area. It occurs from February to November, and reaches its highest density during the spring bloom in February or March. More than a hundred different clones were individually isolated from plankton samples collected over more than one year. Allellic diversity within the population was confirmed by analyzing several microsatellite loci. Great genetic variation indicates that sexual reproduction is important in the population. The high genetic diversity of the population may be a prerequisite for its extensive occurrence during variable seasonal conditions. Sequencing of conserved parts of the genome (LSU rDNA) also displayed a range of genetic variation, whereas morphological features examined in the electron microscope did not vary.

PO.08-05
Gyrodinium fissum: harmful species or new biotechnological object?

Session: PO.08 - Toxicology

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The dinoflagellate *Gyrodinium fissum* (Gf) is poorly studied alga. Some authors regard it as red tide organism; others as non-toxic object of crustacean feeding; Gf biocidal activity has not been properly studied. We exposed lackey and brown-tale moths, fall webworm and Colorado potato beetle (Cpb) to the effects of Gf. The leaves were treated with a Gf culture and fed to the insects. Gf demonstrated various inhibitory effects. (1) Repellent action: lackey moth larvae did not feed and gathered in the substrate and jar walls. (2) Long-term deterrent action with residual consequences: the larval nutrition fell considerably (3.0-5.0% of control). (3) A short-term deterrent action: brown-tale moth larvae. (4) Inhibition of growth: developmental lag of larvae of lackey moth

(50.0%), fall webworm (29.2-68.0); the larvae and imago in Cpb (48.5-63.5% and 15.4-16.1%). (5) Dysfunction of metamorphosis: defects of pupation and imago forming; treatment of Cpb eggs caused elimination of eggs (56.3%) and hatching larvae (32.6%). (6) Mortality during 10-20 days: in lackey moth (95.0%), fall webworm (100.0%) and Cpb (84.4-100.0%). Histological examination demonstrated the degradation of organs and tissues. Gf can be propagated in controlled cultivation; its toxicity is selective: it is not toxic to non-target objects: Gf can be used in biological pest control.

PO.10-37
Ecological and physiological studies of *Dinophysis* spp. during an upwelling-downwelling cycle in Ría de Pontevedra (NW Spain)

Session: PO.10 - Ecophysiology & autecology

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During a two-week multidisciplinary cruise in the Ría de Pontevedra in June 2005 (HABIT 2005) aimed at identifying and characterizing thin layers under stratified conditions, and to study the population dynamics of *Dinophysis* species, biological observations were made in an effort to define the physiological status of each species. Viability assays, percentages of vacuolated specimens, positions of the nuclei, as well as polysaccharide deposits and morphometric measurements were made on live samples. Indications of apparent mixotrophy in *Dinophysis rotundata* and *D. acuminata* were detected by both *in situ* autofluorescence and by using the fluorochrome Sybr-Green. Further experiments are in progress involvog laboratory incubations of isolated single cells of *D. rotundata* and *D. acuminata* in multiwell plates, using a cryptophyte as food, and with a new class of model protein particles labelled covalently with fluorochromes as model particles for the determination of grazing rates.

PO.05-30
Yessotoxin contamination: the first report from Portuguese shellfish

Session: PO.05 - Toxin analysis

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Several Portuguese shellfish species were screened for the presence of yessotoxin (YTX) using a newly developed immunoassay kit, sensitive to a wide variety of YTX analogues (YTXs). Shellfish samples were collected at Aveiro and Formosa Lagoon, and at the Algarve offshore during the summer/autumn 2005. In lagunar species, YTXs were detected in the following at decreasing concentrations: *Mytilus galloprovincialis* >> *Cerastoderma edule* > *Ruditapes decussatus* = *Venerupis pullastra* = *Solen marginatus*, but not in *Crassostrea* spp. In offshore species, YTXs were detected in the following at decreasing concentrations: *Spisula solida* > *Donax* spp = *Chamelea gallina*. Some of the mussels samples collected in the north of Portugal (Aveiro), as well as the south (Formosa), showed levels around the current EU regulatory limit of 1 mg/kg shellfish meat, not exceeding 1.2 mg/kg. A prolonged persistence of YTXs was observed in all three areas studied. At Aveiro Lagoon it was possible to associate temporary increases in YTX's contamination with the presence in the plankton of *Protoceratium* spp. and *Gonyaulax spinifera*, but not with *Lingulodinium polyedrum*. This is the first report of contamination of bivalve molluscs from Portugal with YTXs.

PO.15-33

Minimizing economical losses with 'real-time' HAB surveillance

Session: PO.15 - Monitoring

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Cyanobacterial blooms covering almost the entire Baltic Sea is a normal feature in July-August. For the tourism industry at the island of Öland, SE Sweden, the economical losses during the summer of 2005 amounted to16-21 million euros. As remote sense satellite images have a low resolution, from the pictures of the blooms it looks like that all Öland beaches are covered with decomposing algae. In reality, these blooms rarely affect the western side of the island. By having an intensive daily real-time surveillance of the algal accumulation on the beaches, with quick transfer (within hours) of this information to the public, it is possible to get the tourists back, showing that on almost all days of the two months of blooms, most beaches are fit for bathing. The 'real-time' monitoring is be done by volunteers (pensioners, youth, camp-site workers, etc.) who get the necessary training at the University of Kalmar. Among the skills to be learned by the volunteers are harmful algal species identification and quantification, analyses of cyanobacterial pigments, temperature, salinity, etc. The results for the pilot study (July-August) will be presented.

PO.10-31

Using quantification of gene expression to investigate the initiation phase and dynamics of *Alexandrium catenella* blooms (Dinophyceae)

Session: PO.10 - Ecophysiology & autecology

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Initiation is a critical phase of harmful algal blooms that has been overlooked in field investigations carried out for understanding and predicting their occurrence, for two reasons. First, when toxic cells are detected in phytoplankton by conventional monitoring methods, their concentration is already relatively high (ca 100 cells/L) and environmental conditions are likely changing with respect to the initiation conditions that triggered the cell proliferation. Second, when a cell is observed, its physiological status remains unknown: is this cell actually in growth phase? To get closer to the initiation period of HAB, new tools are required which are more sensitive, specific, and able to give information on cell activity and proliferation dynamics. In the framework of our program investigating *Alexandrium catenella* blooms in the Thau Lagoon (French Mediterranean), we are developing molecular assays to analyze gene expression (mRNA quantity per cell) using reverse transcription and quantitative PCR. We have selected two target genes coding for the RubisCO enzyme (performing carbon fixation and reflecting cell metabolic activity) and the PCNA (proliferating cell nuclear antigen) protein involved in cell division. To test these assays, we will present variations of gene expression analyzed in various experimental conditions.

PO.06-22
Effect of selenium *on Pseudo-nitzschia seriata*

Session: PO.06 - Population dynamics

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The laboratory culture of toxigenic pennate diatoms of the genus *Pseudo-nitzschia* often gives lower growth rates. This is inadequate for ecophysiological studies and for studies involving acid domoic quantification or web-transfer. It is suggested in the literature that addition of extra silicon and selenium is beneficial for growth of diatom cultures, such as *Pseudo-nitzschia* sp. In this work we evaluated the effect of selenium on *Pseudo-nitzschia seriata* growth rates and also on f/2 medium features such as pH, salinity and conductivity, over 15 days at 10 ºC. Addition of selenium, at concentration of 1.29 mg Na_2SeO_3 /L, caused a slight increase of the growth rate and pH. In contrast, significant differences were found in conductivity and salinity between culture mediums with and without selenium. No domoic acid production was detected by HPLC.

PO.14-05
Flow cytometry in conjunction with dual staining assesses viability of *Microcystis* cells after exposure to bacteria

Session: PO.14 - Mitigation

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Flow cytometric (FCM) technique was used to assess viability of *Microcystis* cells after exposure to *Bacillus mycoides* B16. The FCM analysis involved assessment of two cellular functions, esterase activity and membrane integrity, after dual staining with Fluorescein diacetate (FDA) and Propidium iodide (PI). The dual staining of *Microcystis* cells revealed different cell populations: living, membrane compromised and dead cells. The results show that after 2 h of incubation with *B. mycoides* B16 the population of dead *Microcystis* cells was 12%, membrane-compromised cells was 80% and live cells was 8%. The demography of *Microcystis* cells changed after 6 d of incubation, with population of dead Microcystis cells was 51%, those with membrane- compromised cells was 34% and live cells was 15%. In bacteria treated samples the Microcystis biomass was reduced by over 95% whereas with the control samples the biomass increased by over 1000%. The biocontrol agent B mycoides B16 appeared to cause Microcystis cell lysis resulting in cells with compromised membranes with a SEM micrographs signature equivalent to samples that have been lysed with copper sulphate, a known algicide. B. mycoides B16 could therefore be considered as a potential biocontrol agent for the control of harmful algal blooms

PO.13-43
National report of red tides (HABs) in China 2001- 2005

Session: PO.13 - Regional events

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Red tides (HABs) is an important environmental issue in Chinese coastal areas. From the first record of a red tide in 1933, more than 800 red tide events have been recorded in China. In 2001-2005, China witnessed 453 cases of red tides, which covered an area of 93.26 thousand square kilometres totally. Most red tides are located in the East China Sea. Results of monitoring indicate that red tides frequently occur in May/June. 62% of the red tides occurred during this period, representing 83% of the area. Dominant red tide organisms were *Prorocentrum dentatum*, *Skeletonema costatum*, *Noctiluca scintillans*, *Mesodinium rubrum* etc. Poisonous and harmful species like *Karenia mikimotoi*, *Phaeocystis sp*. etc. have increased in recent years. Land-based pollution and eutrophication is suggested to be the main reason causing red tides. Careful monitoring increases the discovery and control of red tides significantly, and emergency response systems play an important role for mitigating the impact of the red tides.

PO.01-21
Occurrence of motile cells of a *Gymnodinium* species, belonging to the *Gymnodinium catenatum* group, in the western Baltic Sea

Session: PO.01 - Genetics

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Since 2003 motile cells of the *Gymnodinium* have been detected in the coastal waters of Schleswig-Holstein within the context of the intensive phytoplankton monitoring (Early Algae Detection System) during the summer months. The species usually appears as vegetative single cells but also 2-cell-chains were observed in the samples. Data show that this *Gymnodinium* did not appear before 2003 and until now there has been no records of motile vegetative cells from the water column of other areas in the Baltic Sea.
The reason may be its low cell numbers but it may also indicate recent changes of environmental conditions, which probably promote the germination of cysts from sediments.

PO.15-31
Field plankton observation: equipment and techniques

Session: PO.15 - Monitoring

S Hall, SM Conrad, SM Etheridge, JR Deeds

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A small, hand-held field microscope permits plankton observations in near real time- a few minutes from net tow to observation. This in turn permits sampling to be directed by what one sees, rather than having to wait until samples are returned to the lab for examination before one knows what was present in the field. Any of several readily available digital still or video cameras can be coupled to the microscope, allowing field observations to be documented. This equipment will be demonstrated during the conference. Please check the bulletin board for announcements of times and locations.

PO.14-04
Efficacy of three commercial ballast water biocides against vegetative microalgae, dinoflagellate cysts and bacteria

Session: PO.14 - Mitigation

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Inclusion within the IMO Ship Ballast Water Convention of a microbial treatment standard has rekindled interest in chemical biocides to combat ballast water mediated aquatic invasions. We examined efficacy of three commercial ballast water biocides, SeaKleen®, Peraclean® Ocean and Vibrex®, using vegetative microalgae, dinoflagellate cysts and bacteria as test organisms. Peraclean® Ocean could effectively inactivate dinocysts of *Gymnodinium catenatum*, *Alexandrium catenella* and *Protoceratium reticulatum* at 400 ppm, control bacterial growth at 125-250 ppm, and eliminate vegetative dinoflagellate cells at 100ppm. It was biodegradable within 2-6 weeks. SeaKleen® did not inactivate cysts of *A. catenella* at 5 times recommended dose (10 ppm), exhibited poor bactericidal properties (100-200 ppm required), and poor biodegradability (assessed through impact on motility of vegetative microalgae). The chlorine dioxide biocide Vibrex® is not a suitable ballast water treatment due to the need for hydrochloric acid as activator, however it was the most effective against bacteria (complete inhibition at 15 ppm). The performance of biocides was adversely influenced by low temperatures (6 vs 17 °C), light vs dark conditions, and incidence of humus and sediments. Routine applicability of active substances is limited by concerns as to cost, biological effectiveness, ship crew safety and residual toxicity of discharged ballast water.

PO.10-10
Vertical distribution of two potentially toxic *Dinophysis* species (Dinophyceae) in the northern Baltic Sea

Session: PO.10 - Ecophysiology & Autecology

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The potentially toxic dinoflagellates *Dinophysis acuminata* and *D. norvegica* are dominant among *Dinophysis* species in the Baltic Sea. We investigated their vertical distributions in the open northern Baltic proper and western Gulf of Finland. *Dinohysis acuminata* was considerably more abundant than *D. norvegica*, especially in the Gulf of Finland. *Dinophysis norvegica* was more frequent and occurred in higher abundances in the northern Baltic proper. It occurred in a narrower temperature and salinity range and may need a more stable water column to develop abundant

populations.
High abundances of *D. acuminata* were usually observed in the illuminated layer above the thermocline or at the thermocline when it was within the euphotic zone and coincided with a nutricline. *Dinophysis norvegica* resided at the thermocline even when it was below the euphotic zone and even though no apparent increase in nutrients was observed. *Dinophysis acuminata* and *D. norvegica* appeared to be vertically separated when co-occurring; this was observed even when both species formed maxima in the top 10 m.

PO.05-24
Development of an enzyme-linked immunosorbent assay (ELISA) for detection of paralytic shellfish poisoning toxins (PSP)

Session: PO.05 - Toxin analysis

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A rapid and sensitive ELISA for detection of PSP in shellfish was developed, using a monoclonal antibody (GT-13A), which was previously confirmed to react specifically to various kinds of standard PSP, and was evaluated for PSP monitoring. The ELISA kit, consisting of the antibody (GT-13A) solution, anti-mouse IgG-antibody coated plate, decarbamoylsaxitoxin-peroxidase conjugate solution, washing solution and stopping solution, was made for practical use. By the ELISA kit, at least 40 samples can be measured within 40 min, and the detection limit of PSP was 0.2 nmol of PSP (gonyautoxin 2/3) per g of shellfish. More than 1300 specimens of samples were collected from various areas of Japan in 2002-2004 and were measured by the ELISA. Good correlation was observed between the ELISA values and those of mouse bioassay (MB). In the ELISA, because of the high sensitivity, small amounts of PSP were detected from 461 of 611 samples which gave negative results by MB. The ELISA is considered to be a useful tool for PSP monitoring of shellfish.

PO.13-29
About toxic cyanobacteria in Tunisia's fresh water

Session: PO.13 - Regional events

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A survey on cyanobacteria has been conducted in aquatic environments in Tunisia (marine water, dams, thermal waters, etc.). Species were named and critical periods identified. Species composition, biomass and dynamics of the cyanobacteria depended on many factors. Besides the trophic status of a particular water mass, weather seasonality also plays an important role. Excessive growth of cyanobacteria and strong dominance of *Microcystis aeruginosa* reflected an exceptionally long hot dry period.

PO.01-18
Phylogeny and biogeography of *Prorocentrum donghaiense*

Session: PO.01 - Genetics

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This research represents the first comprehensive study of the phylogeny and biogeography of *P. donghaiense* from the East China Sea, Korean coast and the East Pacific (South America).
The 18S ribosomal RNA gene was used as a phylogenetic and biogeographic indicator. The four *P. donghaiense* strains were positioned i the same clade on the phylogenetic tree. The bootstrap value of 93.2% between the West Pacific and the East Pacific strains indicate that the populations originated from the same ancestor. *Prorocentrum donghaiense* strains from the East China Sea and Korean coast are closely related to one another, which suggests that *P. donghaiense* from the East China Sea may be native to the Korean coast. From the high degree of homogenity within the globally distributed clade of *P. donghaiense*, we conclude that *P. donghaiense* from the East Pacific may be the origin of these populations. The clade of *P. donghaiense* is closely related to the clade of *P. minimum*.

PO.15-30
Blooms of cyanobacteria in the Baltic Sea 1997-2006 detected using satellite – the phosphorus connection

Session: PO.15 - Monitoring

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Summer blooms of nitrogen-fixing cyanobacteria are regular phenomena in the Baltic Sea. In the past years strong and widespread blooms have caused environmental concern due to nuisance, toxicity and the increased nitrogen input. The most abundant toxic species, *Nodularia spumigena*, can pose a threat to small animals and children. Based on satellite sensor AVHRR (Advanced Very High Resolution Radiometer), SMHI has a compiled time series of surface accumulations of cyanobacterial blooms during the period 1997-2006. Results indicate large inter-annual variations that could be connected to periods with oxygen free conditions in the bottom water in the Baltic Sea and the resulting release of phosphorus from the sediments to deep water. During inflow of oxygen rich and saline water from the Kattegat, deep water can be moved closer to the surface and eventually the water with high concentrations of phosphorus is transported into the surface layer due to wind mixing. Since most cyanobacteria have the ability to fix nitrogen, the main limiting factor is the amount of available phosphorus. Other factors that also influence the occurrence of strong blooms are the sea surface temperature, wind conditions and stratification.

PO.05-37
Analysis of phycotoxins in hand-picked plankton cells by micro-column liquid chromatography-tandem mass spectrometry

Session: PO.05 - Toxin analysis

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The toxin concentration and profile present in plankton can vary considerably between different geographical areas and even within a region and between seasons due to the presence of different species and strains. Traditional methods of analysis require substantial sample sizes (e.g. millions of cells) making it difficult to attribute the presence of toxins or variations in toxin profiles to individual species. This paper will present a new method based upon a micro- sampling and extraction procedure coupled with micro-column liquid chromatography-tandem mass spectrometry (LC-MS). This sensitive method makes analysis of toxin content within single cells from both cultures and field samples, a possibility. LC-MS analyses of spirolides, pectenotoxins and yessotoxins from intact cells picked from field samples are provided. Variations in toxin profiles were observed in single cells of *A. ostenfeldii* from a sample collected at Ship Harbour, where we have observed considerable variability in toxin profile in previous years. In clonal cultures of *A. ostenfeldii* (AOSH1) similar toxin profiles were observed, however there was a strong correlation between cell size and toxin concentration.

PO.16-08
Significance of benthic recruitment on the dynamics of harmful phytoplankton blooms in the tropics

Session: PO.16 – Life cycles

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Many harmful marine microalgae have a benthic resting-stage as part of their lifecycle. Although phytoplankton blooms are major events in aquatic systems, the importance of benthic resting stages in seeding planktonic blooms is still unclear. Using mesocosms, we tested the influence of benthic vs. planktonic inocula on the development and taxonomic composition of tropic phytoplankton communities. The experiment revealed that the type of inoculum influenced bloom development and community structure. Taxa not known to form resting stages, such as *Pseudo-nitzschia* spp. and *Skeletonema tropicum* were very abundant in the plankton inoculated mesocosms. Species composition was different when seeded by cells from the benthos. Resting-stage forming species, such as representatives of the genus *Thalassiosira*, thrived in the sediment inoculated mesocosms. These species displayed two temporally separated density peaks in mesocosms inoculated with both plankton and sediment. Most probably the second peak was a consequence of germinated resting stages. Our results indicate that benthic resting stages provide an important source for some species. The introduction of benthic resting stages to surface waters can greatly influence species composition of the plankton, and therefore it is important that studies of plankton blooms include life history stages from both the sediments and water column.

PO.14-11

Growth control of toxic microalgae by weak voltage and weak current

Session: PO.14 - Mitigation

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It is well known that the weak voltage and current in the nervous system influence the physiology of animals. On the other hand, little is known about the influences of voltage and the current on the physiology of algae and plants.
The use of a slight voltage and current was reported as adhesion prevention of the barnacle by Inoue *et al*. (Fisheries Engineering in Japan 2004 Vol. 41 No1. pp. 47-52).
With a relatively low voltage (3 to 12V) movement to the electrode (30 cm between the electrodes) and whitening of the toxic microalgae was observed.
Intermittent and intersection voltage and current supply have possible effects on the growth of the algal cell.
The voltage and the current supply have influence on surfacing and sedimentation of the algae. Effects of daytime and nighttime was also seen.
The electrical stimulations may cause disturbance and confusion of electrical potentials of the cell films and thereby influence mineral nutrition, oxygen and carbon dioxide intake of the cells.

PO.04-11
Uptake, metabolism and loss of clay-flocculated brevetoxins in a surface deposit-feeding clam

Session: PO.04 – Food chains

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Blooms of the brevetoxin-producing *Karenia brevis* in the Gulf of Mexico cause fish kills, food poisoning and respiratory irritation in humans. Sedimentation of toxic cells following clay application could reduce toxin incorporation by commercially important suspension-feeding bivalves and direct public health impacts, but may potentially lead to brevetoxin (PbTx) accumulation by benthic deposit-feeders. The goal of this study was to determine whether deposit-feeding could provide a pathway of toxin transfer from deposited clay-*K. brevis* aggregates. We investigated PbTx uptake, metabolism and detoxification kinetics in a deposit-feeding, tellinid clam exposed to clay-deposited brevetoxins. We demonstrate that brevetoxins can be rapidly accumulated by deposit-feeding from sedimented *K. brevis* cells (exceeding the regulatory level of 0.8 mg PbTx g^{-1} within ~12h as determined by ELISA). LC-MS analysis showed that PbTx-2, the dominant toxin in the clay/cell layer, was rapidly transformed into PbTx-3 and its cysteine derivatives in clam tissues. Detoxification of tissues following deposit-feeding occurred but toxicities remained around the regulatory level for 15 days. This toxicity was due largely to the persistence of the more potent PbTx-3-cys metabolites in tissues. Deposit-feeding clams do not pose a direct threat to humans but may provide a pathway for brevetoxin food web transfer.

PO.05-09

In vitro interactions between several species of harmful algae and hemocytes of bivalve molluscs

Session: PO.05 - Toxin analysis

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Harmful algal blooms (HABs) can have noxious and sublethal impacts on shellfish. The northern quahog (= hard clam), *Mercenaria mercenaria*, can experience blooms of several HAB species, including *Prorocentrum minimum*, *Heterosigma akashiwo* and *Alexandrium fundyense*. To understand the possible roles of the hemocytes in bivalve responses to HABs, and how the algal cells are affected by these responses, in vitro tests of interactions between those harmful algae and hemocytes of *M. mercenaria* were carried out. We measured possible differences in hemocyte parameters attributable to harmful algae and also the effect of hemocytes on the algae themselves. Using microscopic and flow-cytometric observations, changes in hematology and physiology, including cell concentration, phagocytosis, adhesion and oxidative burst response of hemocytes were determined. Changes in the physiology and the characteristics of the algal cells including mortality, size, chlorophyll fluorescence and internal complexity were also determined. The results show a species-specific response of the hemocytes depending upon the harmful algae to which they were exposed. Thus, in vitro tests allow a better understanding of the role of the hemocytes and the hemolymph in the defense mechanisms in molluscan shellfish to harmful algal cells.

PO.05-27
Newly discovered brevetoxin oxidation products in marine aerosol: assessing potential public health impacts

Session: PO.05 - Toxin analysis

Michael S Henry[1], RH Pierce[1], PC Blum[1], KL Lemkau[1], B Kirkpatrick[1], SE Osborn[1], YS Cheng[2], Y Zhou[2], LE Fleming[3], LC Backer[4], S Plakas[5], A Abraham[5], R Dickey[5], A Reich[6], A Bourdelais[7], J Naar[7], DG Baden[7]

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The toxic dinoflagellate, *Karenia brevis*, produces polyether neurotoxins (brevetoxins or PbTx) that cause massive aquatic animal mortality and neurotoxic shellfish poisoning (NSP). A unique characteristic of *K. brevis* blooms is the associated airborne brevetoxin component that results in severe respiratory problems in exposed populations. This study was undertaken in collaboration with human exposure studies to determine the composition of aerosolized brevetoxins to which beachgoers are exposed during a

K. brevis bloom. Brevetoxins and related degradation products were extracted from water collected along the shore and from marine aerosols. Water samples were further processed to separate the toxins contained inside (intracellular) as well as outside (extracellular) the *K. brevis* cells. Oxidation and/or hydrolysis products of PbTx-1, -2, -3 and -7 were found only in extracellular water and in aerosols and at levels comparable to the parent brevetoxins. These compounds have been previously described in laboratory culture, natural bloom water, and shellfish, but have not been described in marine aerosols to which people and other mammals are exposed. Knowledge of these compounds and their mechanisms of aerosolization will provide critical information regarding the cause and effect relationship on human and animal health during red tide aerosol exposure.

PO.05-45
Development of a screening method for cyanobacterial toxins

Session: PO.05 - Toxin analysis

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Mass developments of toxic cyanobacteria in fresh waters in combination with health risks and several lethal poisonings of animals as well as human beings have been documented. The most important group of cyanobacterial toxins are hepatotoxins, dominated by microcystins in addition to nodularins. Other cyanobacterial toxins are anatoxin-a, paralytic shellfish poisoning toxins and cylindrospermopsins. Due to the cases of human intoxication frequently connected with cyanobacterial blooms, governmental institutions established control programmes to ensure that toxins do not reach the consumer. Therefore, many methods have been developed for specific target compounds of most of the toxin groups. These methods have their benefits and are in use at numerous analytical laboratories. However, the application of specific methods depends on the knowledge of the presence of a toxin-producing organism. If this information is lacking toxin analysis can be very tedious. Recently, we have developed a liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) method, which qualitatively monitors various typical representatives of all known classes of cyanotoxins, including even unknown derivatives. This method uses diagnostic mass fragments for the detection of characteristic compounds of the different classes and thus allows monitoring a large set of unspecified cyanobacterial samples for the presence of any kind of cyanotoxins.

PO.15-24
Quantification of epibenthic communities, including toxic dinoflagellates, in different green macroalgal substrates in Ria de Aveiro (Portugal)

Session: PO.15 - Monitoring

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The coastal lagoon ‘Ria de Aveiro’ is a complex system of channels, marshes and puddles that support the growth of a variety of macrophytes. In sheltered areas extensive masses containing mixtures of green algae can be found, among which three main types can be distinguished: those predominantly made up of species of *Ulva* (including *Enteromorpha*), entangled filaments of *Cladophora*, and elongate masses of *Chaetomorpha linum*. High numbers of microscopic algae can be found attached to these substrates or dwelling close to the macroalgal surfaces, forming diverse epibenthic communities. Attached diatoms make up the greatest abundance of epibenthic organisms, especially in more exposed areas, but a variety of dinoflagellates, including toxin-producing species, are regular members of the community in sheltered areas. The composition of these communities in the three different types of green algal masses was examined every fortnight in two sheltered localities, using a quantification method that involves the forced detachment of the organisms from a known weight of macroalgal mass, followed by counting in sedimentation chambers. Differences in the abundance of epibenthic species in the different types of substrates were noted and may be useful for directing efforts for finding toxic species in other areas of this extensive lagoon.

PO.04-08

An individual-based model simulates the effects of brown tide on larval recruitment of hard clams

Session: PO.04 – Food chains

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Experimental data were used to modify an individual-based model that simulates growth, development, and metamorphosis of hard clam, *Mercenaria mercenaria,* larvae to include the effects of brown tide (*Aureococccus anophagefferens*). The coupled model was applied to predict brown tide effects on clam larvae in a USA estuary (Great South Bay, New York) where blooms of varying magnitude have occurred for 20 yrs coincident with the period of clam spawning and larval development. Initial simulations showed that temperature and food quantity variations produce small changes in overall larval survivorship, whereas changes in food quality (especially reduced lipid content) have the largest effect on larval survival. Simulations that include brown tide effects show inhibition of *M. mercenaria* larval growth that is observed in laboratory experiments with a toxic *A. anophagefferens* strain. When brown tide occurs over 2 wks of development, followed by a period of no exposure, the model predicts considerably reduced postlarval recruitment success. The simulated genetic structure of the population shows that brown tide affects the genotypic characteristics of hard clam populations. For example, simulations show that brown tide exposure selects against fast growing larvae derived from large eggs that attain competence without the necessary lipid reserves to sustain metamorphosis.

PO.10-32
Culture and ichthyotoxicity of the red tide dinoflagellate *Noctiluca scintillans*

Session: PO.10 - Ecophysiology & autecology

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Noctiluca scintillans red tide frequency and distribution has increased in Tasmanian waters, Australia since the first sighting in 1994. This study maintained the first Australian *Noctiluca* culture for 8 months during laboratory experimentation. Cultures were kept at 17 °C and 28 ‰ salinity to balance *Noctiluca* grazing and growth of the dinoflagellate prey, *Gymnodinium catenatum*. Specific growth rate increased with temperature from 0.24 at 12 °C to 0.47 at 23 °C and decreased with increasing salinity from 0.6 at 20 ‰ to 0.33 at 30 ‰. Culture growth rates support field evidence of frequent summer coastal blooms and survival of overwintering estuarine seed populations.
Field sampling investigated the potential fish killing mechanisms associated with surface slicks, which severely threatened Tasmanian aquaculture farms in 2002. Red tides exhibited higher cell ammonia (NH_3) concentration than water column populations. Red tide populations produced seawater NH_3 levels fatal to salmon and liberated a high proportion of free fatty acids in comparison to blooms. Polyunsaturated fatty acids included eicosapentaenoic acid with a history of toxicity and docosahexaenoic acid common to dinoflagellates. Saturated fatty acid eicosanoic acid was identified as a potential *Noctiluca* biomarker.

PO.10-24
Nutrient physiology of *Prorocentrum donghaiense* Lu from Eastern China Sea

Session: PO.10 - Ecophysiology & autecology

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This study investigated nutrient requirements, physiological response to nutrient variations and adapting strategy to low nutrients in *Prorocentrum donghaiense* Lu, a key HAB species, which is widespread in the East China Sea. *Prorocentrum donghaiense* is able to utilize various inorganic and organic nitrogen and phosphorus sources as sole nitrogen and phosphor source for cell growth and biosynthesis. Various enzymes play important roles in nutrient uptake and assimilation. Comparative protein profiles of *P. donghaiense* under nutrient replete and N or P limited conditions indicate that cells grown under N limitation had more potential to form blooms than P limited cells. Several protein groups related to nutrient status of cells were identified, and some proteins could be used as potential nutrient indicator. Time series of protein profiles of *P. donghaiense* under long-term low nutrient growth conditions showed that some proteins remained relatively constant and played important roles in maintaining *P. donghaiense* population at low nutrient conditions. Our results indicated

that *P. donghaiense* has a numbver of nutrient-utilizing systems and low nutrient-adapting mechanisms, which may aid during competition with other phytoplankton species under low nutrient environment.

PO.13-48
Pseudo-nitzschia and ASP in the northern Adriatic Sea

Session: PO.13 - Regional events

G Honsell[1], C Dell'Aversano[2], F Vuerich[1], S Sosa[3], L Tartaglione[2], A Tubaro[3]

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The presence of domoic acid in mussels grown in Northern Adriatic Sea has been recently reported. Although *Pseudo-nitzschia* species have been abundantly found in phytoplankton, forming spring-summer and autumn blooms, taxonomical information on this genus including domoic acid producers are generally lacking. For this reason, a survey on *Pseudo-nitzschia* was carried out in the Gulf of Trieste in 2005 based on phytoplankton qualitative and quantitative analysis, taxonomical identification by TEM and ASP toxins analysis. The most abundant species were *Pseudo-nitzschia calliantha* (54420 cells/L) and *Pseudo-nitzschia delicatissima* (20840 cells/L), as previously observed also in Southern Adriatic Sea. Some clones of these species are known to produce low amounts of domoic acid, such as 0.007-0.221 pg/cell and 0.005-0.12 pg/cell, respectively. Consequently, the concentration of amnesic toxins both in natural phytoplankton net samples and in seawater was under the detection limit of both Biosense ASP ELISA (10 pg/ml) and HILIC-MS (3 ng/ml in MRM positive ion mode transition at m/z 312>266). These results confirm that *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia delicatissima* are the most common *Pseudo-nitzschia* species in the Adriatic Sea, although their involvement in ASP toxins production has to be demonstrated.

Thanks to Friuli Venezia Giulia Government for funding this research.

PO.16-07
Alexandrium cysts in Puget Sound, Washington, USA

Session: PO.16 – Life cycles

RA Horner[1], CL Greengrove[1], JR Postel[1], JE Gawel[1], KS Davies-Vollum[1], A Cox[2], K Sorenson[1], J Hubert[1], S Hoffer[1], J Neville[1], BW Frost[1]

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Paralytic shellfish poisoning is often common in shellfish throughout much of Puget Sound, but little is known about the distribution of either the motile cells or cysts of the major causative species, Alexandrium catenella. A survey of 32 sites in the spring of 2005 showed that cysts are present in surface sediments throughout Puget Sound, being most abundant in the northern and central areas. Sequim Bay in the north with 200 cysts/ml sediment and Quartermaster Harbor in the central part with 12,000 cysts/ml sediment have the highest abundances. Elsewhere where cysts were found, numbers ranged from < 10 to about 100 cysts/ml. There appears to be no correlation between sediment grain size and cyst abundance. Cyst abundance

correlations with sediment metal concentrations and total organic carbon are being investigated. Cyst germination studies, still in progress for Quartermaster Harbor, suggest the presence of an endogenous clock.

PO.10-23
Identification and characterization of cell surface proteins in the toxic dinoflagellate *Alexandrium catenella* DH01 using epifluorescence, immunoproteomic approach and MS-MS

Session: PO.10 - Ecophysiology & autecology

Xu Guang Huang[1], Da-Zhi Wang[1], Leo Lai Chan[2], Hua-Sheng Hong[1]
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Alexandrium catenella DH01 is a key harmful algal species along the China Sea. This study developed cell surface specific antiserum using 0.5% paraformaldehyde (PFA)-fixed cells as antigen, and investigated cell surface proteins using a combination of 2-DE and immunoblot analyses. The results showed that PFA-fixed WCA-derived antiserum specifically recognized weakly bound cell surface proteins (CSPs) in *A. catenella* DH01. Using these theca-specific antisera, about 42 abundant cell surface associated antigenic spots were identified on the 2-DE immunoblots. Nine of the most abundant 42 proteins identified by this approach gave positive identification of protein orthologues in the protein database by MS-MS analysis. Peridinin-chlorophyll a binding protein (PCP) appeared to form the most prominent spots on the cell surface. In addition, four putative transporter proteins were identified by this immunoproteomic approach. They are involved actively in the transport of a broad range of substances across the membranes, as well as five groups of cell surface associated proteins.

PO.01-10
Molecular detection and diversity of *Pseudo-nitzschia* populations from the North American West Coast

Session: PO.01 - Genetics

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University of Washington, SEATTLE, United States of America

The diatom *Pseudo-nitzschia* produces domoic acid, which has led to closures of shellfish harvests along the US Washington coast and in the Puget Sound estuary. We developed automated rRNA intergenic spacer analysis (ARISA) to rapidly identify different species of *Pseudo-nitzschia*. In silico analyses were used to design *Pseudo-nitzschia* specific PCR primers that amplify a polymorphic region of the internal transcribed spacer 1 (ITS1) from at least thirteen *Pseudo-nitzschia* species over a broad geographic range, including species from Europe, Tasmania, Vietnam, and Central and North America. These primers were used to generate clone libraries from environmental samples. Amplicon length remained consistent for a species, though intraspecific sequence variability was detected. For example, 68 *Pseudo-nitzschia pungens* clone sequences were generated, all with an amplicon size of 142 base pairs,

and thirteen *P. pungens* sequence types were detected (with up to 3% differentiation). We used ARISA to determine *Pseudo-nitzschia* ITS1 amplicon sizes- and thus species composition- in a variety of environmental samples, including during toxic events. During a domoic acid closure in Washington, ARISA was used to identify the most abundant type of *Pseudo-nitzschia* present as *P*. sp., with an amplicon length of 233, which does not correspond to anything in Genbank.

PO.16-13
Spatial distribution of dinoflagellate cysts in sediments of the Yellow Sea

Session: PO.16 – Life cycles

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We analyzed 33 sediment samples from the Yellow Sea by palynological methods to investigate spatial distribution of dinoflagellate cysts. The sampling areas comprised four latitudinal transects, the northernmost located off the Shandong Peninsula of China, and the southernmost off Jeju Island of Korea. Each transect line comprised six to nine stations, spanning the distance between the Chinese and Korean coasts. In total, we identified 26 different types of dinoflagellate cysts. *Gonyaulax scrippsae*, *Alexandrium* spp. (ellipsoidal type) and *G. spinifera* were dominant at all stations surveyed. The latitudinal distribution trend showed that cyst concentrations along the inner two transects were much higher than those along the outer two transects. Within each transect, cyst concentrations in the offshore central areas reached the highest values and gradually decreased toward both the Chinese and Korean coasts. Overall, cyst concentrations were markedly elevated in the offshore central Yellow Sea areas and gradually decreased outward in all four directions. This concentric cyst distribution pattern was consistent with hydrographic features such as circular current systems, sedimentary properties and water depth of the Yellow Sea.

PO.14-08
Differences in susceptibility of harmful raphidophytes and dinoflagellates to algicidal bacteria isolated from coastal sea and seaweed beds

Session: PO.14 - Mitigation

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Growth and survival of harmful algae are largely influenced by algicidal bacteria in coastal ecosystems. The susceptibility of 3 raphidophytes (*Chattonella antiqua*, *Heterosigma akashiwo* and *Fibrocapsa japonica*) and 2 dinoflagellates (*Heterocapsa circularisquama and Karenia mikimotoi*) was investigated for 411 bacterial isolates from seaweed beds (seaweeds and seawater) of shelving sea bank at Kansai International Airport and offshore in the Seto Inland Sea in August and November 2003. A total of 77 strains exhibited algicidal activities but the range of susceptibility to algicidal bacteria showed great variations. Some bacteria specifically killed only one of the algal species examined, and some killed 2, 3, 4 or all 5 species. The density of the algicidal bacteria was in thousands / mL or higher in seawater of seaweed beds, much more abundant (50 times or more) than offshore. In offshore seawater, algicidal bacteria were associated with particles. In seaweed beds, the same species of algicidal bacteria were detected in particle-associated and free-living fractions of seawater and on the surface of seaweeds. Sequence analyses of SSU rRNA gene revealed that algicidal bacteria belonged to marine *Cytophaga*/*Flavobacterium*/ *Bacteroides* group and α- and γ-Proteobacteria. The isolates with the same sequences to algicidal bacteria did not always show algicidal activities.

PO.14-16
Effects of alternate current on growth of and damage to toxic microalgal cells

Session: PO.14 - Mitigation

Ayaka Ishiguro, Shin Takano, Hitoshi Ogawa

Univ. Tamagawa, MACHIDA-SHI TOKYO, Japan

A typical pattern of the alternate current of the commercial electric power is the sine wave. However, there are various wave shapes of the alternating current, e.g. quadrate, triangle, saw shaped, bias, etc. Moreover, parameters such as voltage, current, frequency, distance between electrodes, continuousness and intermittence of the current can be varied. Although the risk for electric shock is higher with alternate current than direct current electricity, the alternate current of low voltages and currents are used for medical treatment.
We observe sometime that microalgae whiten in connection with electric leaks from submerged pumps in backyard ponds. Effects of application patterns of the alternate current on different kinds of algae were investigated. Different effects, e.g. whitening, flaking off, cohesion and the separation, etc. on algae were observed. It showed that the SSB wave of the alternate current causes gathering of the algae on the electrode side. Further research on removal of the toxic substances from collected algae is required.

PO.16-11
Dynamics of *in situ* cyst germination and vegetative population of *Alexandrium catenella* in an embayment, central Japan

Session: PO.16 – Life cycles

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Seasonal variations of *in situ* germination flux of cysts and abundance of vegetative cells of the toxic dinoflagellate *Alexandrium catenella* were investigated in Ago Bay, central Japan, between July 2003 and December 2004. The *in situ* germination flux (cells/m^2/day) was measured using a new device 'Plankton Emergence Trap/Chamber (PET Chamber)' that we recently developed. Germination of the cysts on the sediment occurred continually, ranging from 52 – 1753 cells/ m^2/day, with no seasonal trend. In contrast, the vegetative population showed a bimodal seasonal pattern – a larger bloom from spring to early summer and a smaller bloom from autumn to early winter, although the cell concentration in both blooms were not high (maximum 1.9×10^3 and 1.7×10^2 cells/L, respectively). The size of the vegetative population did not correlate with that of germination but a larger population was often observed when the water temperature was around 20 °C, indicating that bloom development is mainly controlled by the temperature but not by the germination. However, the continuous germination mode of *A. catenella* is advantageous for the vegetative cells to immediately exploit favorable conditions for blooming.

PO.12-01
First record of *Ostreopsis* spp. in Egyptian waters with a description of *O. mediterraneus* n. sp.

Session: PO.12 - Taxonomy and phylogeny
Presentation time: 16:40 - 18:00

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A population of *Ostreopsis* spp. is reported for the first time from Egyptian waters. Macroalgal samples were collected monthly since June 2005 from 5 different sites along the Alexandria coast from less than 1.5m depth and their associated microalgae examined. Populations of two *Ostreopsis* species occurred along the eastern sector of Alexandria: *O. ovata* Fukuyo and *O. mediterraneus* n. sp. *Ostreopsis mediterraneus* n.sp. was abundant and dominant during summer. It was more abundant on the brown algae *Padina* sp. and *Sargassum* sp., less abundant on the red algae *Corallina* sp., *Jania* sp. and *Laurencia* sp. and even less so on the green algae *Ulva* spp. *Ostreopsis ovata* was also found during summer months on the same algal species but at much lower abundance.
*Ostropsis mediterraneu*s n. sp. alternated in dominance with the benthic cyanobacteria *Oscillatoria* spp. and the diatom *Licmophora* sp. Other benthic dinoflagellates recorded at low abundance were *Amphidinium carterae*, *Gymnodinium* sp. and *Prorocentrum lima*. *Ostreopsis mediterraneus* somewhat resembles *O. caribbeanus* but is easily distinguished by its shape, smaller size and the shape of the diagnostic plates.

PO.07-02
Induced development of algal blooms using sewage enrichment

Session: PO.07 - Ecology and oceanography

WA Ismail[1], FY Al-Yamani[1], KS Al-Rifaie[1], DV Subba Rao[2]
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Globally, the frequency of red tides has been on the increase. Some of the algal bloom incidences are attributed to urban pollution and eutrophication. This is true in the waters off Kuwait where 18 potentially harmful algal species exist. A perturbation experiment was conducted, which demonstrated that natural assemblages of phytoplankton from the Kuwait Bay environment could be stimulated and sustained to bloom levels. High levels of algal density (up to 28.9 million flagellates/l, 31.6 million picoplankters/l and 8.9 million other algal cells/l) were attained. Phytoplankton biomass measured in terms of chlorophyll also responded similarly. From this study, it is evident that enrichment with sewage could induce development of algal blooms in microcosms. The magnitude of the blooms and their production characteristics were high. The presence of a variety of phytoplankton species in bloom densities, particularly the dinoflagellates implicated in toxigenic episodes elsewhere, is a matter of concern and demonstrated the existence of a potential for development of toxigenic algal blooms off this coast due to eutrophication.

PO.08-01
Verification of diarrhetic activities of PTX-2 and okadaic acid *in vivo*

Session: PO.08 - Toxicology

Emiko Ito
Chiba University, CHIBA, Japan

Diarrhetic activities of OA and PTX-2 were studied in mouse and rat by oral route.
Stock solutions of OA and PTX2 were prepared with 50 % ethanol and dimethylformamide and diluted with saline before use. At sublethal doses, both toxins produced in mice the severest tissue injuries in the small intestine at 60 min and the maximum fluid accumulation at 90 min. The recovery thereafter was well and rapid. The minimum adverse effect doses for OA and PTX2 were estimated to be 75 µg/kg and 400 µg/kg, respectively. Single administration of OA at 50 µg/kg or PTX2 at 300 µg/kg did not cause fluid accumulation, however, by co-administration they had synergistic effect. The effects of the two toxins were discernible. The OA-induced erosion was characterized by edema, while vacuole formation at epithelial cells was more prominent with PTX2. In contrast to mice, rats were more tolerant to the toxins. Both OA and PTX2 diluted in saline caused intestinal fluid accumulation at 400 µg/kg and over. Use of triolein-oil for OA dilution lowered the minimum effective dose to 200 µg/kg and the use of 2% lecithin-water lowered the minimum effective dose for PTX2 to 300 µg/kg.

PO.01-23
Phylogenetic relationships between *Cochlodinium polykrikoides* populations from Japanese and East Asian coasts

Session: PO.01 - Genetics

Mitsunori Iwataki[1], Hisae Kawami[1], Kazumi Matsuoka[1], Y Fukuyo[2]
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The unarmoured dinoflagellate *Cochlodinium polykrikoides* is responsible for recurrent mass mortalities of fish in East and Southeast Asian countries. In Japanese and Korean coastal waters, blooms of this species occur every summer. To distinguish the populations and their geographical distribution, SSU rDNA and partial LSU rDNA sequences of *C. polykrikoides* collected along the coasts of Western Japan were analyzed and compared to sequences from Korea, Philippines and Malaysia. In the phylogenetic tree constructed by the gamma weighted neighbour-joining method, all strains formed a well-supported monophyletic group within the dinoflagellates. Sequences of all Korean and almost all Japanese strains were identical, while other strains had several base substitutions. This indicates that the dominant population of *C. polykrikoides* from Japanese and Korean coastal waters could be separated from populations inhabiting Southeast Asian countries. Moreover, chain-forming *Cochlodinium sp.* specimens, showing a morphological resemblance to *C. polykrikoides* (i.e. possessing chloroplasts and an eyespot), were also found in this study. This species branched out as a sister to *C. polykrikoides*.

PO.02-02
Genomic characterization of the spirolide-producing dinoflagellate *Alexandrium ostenfeldii* with special emphasis on PKS genes

Session: PO.02 - Genomics

N Jaeckisch[1], G Glöckner[2], H Vogel[3], A Cembella[1], U John[1]
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The dinoflagellate *Alexandrium ostenfeldii* is the only known producer of toxic spirolides. Spirolides are macrocyclic imines that are derived via polyketide biosynthetic pathways - production is therefore almost certainly mediated by polyketide synthase (PKS) genes. At the genomic level, studies on the biosynthesis of protist-derived polyketides have been very sparsely reported. For dinoflagellates, this is due in part to the peculiarities of the dinoflagellate genome in both structural and regulatory respects. Our research focuses on the identification and characterization of genes involved in spirolide biosynthesis, specifically PKS genes. Genomic characterization of *A. ostenfeldii* was conducted by generating an Expressed Sequence Tag (EST) data-bank, based on a normalized and a standardized cDNA library. About 20,000 ESTs were sequenced and compared from two strains of *A. ostenfeldii* (AOSH1 and AOSH2) from Nova Scotia, Canada, which produce distinctive spirolide profiles. Several genes putatively related to toxin synthesis were detected, including genes encoding PKS. Genome size and gene density were estimated via a genomic library analysis. A fosmid library was also generated to detect and further analyse toxin-related genes. Significant insights into the general genomic organisation of *A. ostenfeldii* and the relationship with putative toxin-producing genes were obtained by this multi-dimensional limited genomic characterization.

PO.10-05
Short-term temporal variability of ammonium and urea uptake by *Alexandrium catenella* and *A. minutum* in culture

Session: PO.10 - Ecophysiology & autecology

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In batch cultures of *A. catenella* (2 strains) and *A. minutum* on a diel light cycle, ammonium uptake was estimated by the 15N tracer technique and could be described by Michaelis-Menten kinetics, while urea uptake was related in a linear or sigmoid way to urea concentration in the range 0.1 to 10 µgat N/liter. The maximum uptake rate (Vmax) for ammonium increased over the day (3-6 h time scale) by a factor of 2-7 depending on species and strain. Half-saturation constants for ammonium (KNH_4) did not show significant differences over time, but the KNH_4 for the Olbia strain of A. catenella were lower than those of the Tarragona strain by factors of 2 to 10. Another measure of affinity for ammonium, the initial slope of the uptake rate – concentration relationship, always increased over the daylight period, indicating that those organisms are influenced by the diel cycle through their capacity to absorb ammonium at low concentrations. For urea, the uptake rate at 10 µgat N/l increased over time only for the Olbia strain of *A. catenella* (by a factor of two). Those results are discussed in terms of the contribution of ammonium and urea to the growth of those dinoflagellates.

PO.15-19
The sampling technique greatly affects the toxin content in *Dinophysis* spp. cells

Session: PO.15 - Monitoring

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The toxin content in cells of *Dinophysis* spp. is often measured from concentrated water samples. One reason for this is that *Dinophysis* spp. normally appears in relatively low numbers. In autumn 2005, water samples were collected using a submersible pump. Samples were collected without concentration and with a concentration factor of 500 or 1000, using a plankton net of 20-µm mesh size. Cell number per liter was enumerated and toxin content per liter was measured using LC-MS. The result was striking. By concentrating the samples, at least two thirds of the toxin disappeared. The average cell toxin content of *D. acuminata* in non- concentrated samples was slightly over 100 pg OA/cell whereas concentrated samples only contained about 35 pg OA/cell. The same pattern of toxin loss was also found in cells of *D. acuta* and its content of DTX-1 and DTX-2. These results strongly emphasize that the handling procedure of the cells has a crucial effect on the toxin content in the cell. This has to be considered when evaluating the toxicity of *Dinophysis* and its impact on mussel toxicity.

PO.02-05
Polyketide synthases in protists: a class of their own

Session: PO.02 - Genomics

Uwe John
Alfred Wegener Institut, BREMERHAVEN, Germany

Polyketides (PK) is a structurally diverse class of natural products, including substances of pharmaceutical, industrial and chemical ecological interest. PK express a number of human health-related effects (antibiotic, antitumor, and immunosuppressive agents) and many from marine micro-organisms are potent biotoxins, which are involved in processes such as chemical defence or complex cell communication. They are synthesized by successive condensations of acetate units via activity of enzymes known as polyketide synthases (PKSs). Bacteria, fungi and plants may produce PKS but Type I modular form was formerly known only for bacteria and fungi. DNA sequences were used to identify modular Type I PKS genes within genomes of protists belonging to the evolutionary lineages of alveolates and amoebozoa. We present that the newly sequenced genomes of representatives of other protistan groups, the chlorophytes *Ostreococcus tauri* and *Chlamydomonas reinhardtii*, and the haptophyte *Emiliana huxleyi*, as well as expressed sequence tags (ESTs) of various harmful microalgal species also contain putative PKS genes. Bioinformatic analysis reveals that these protistan PKS genes cluster into a unique clade. These observations provide important insight into the evolution of polyketide metabolic pathways and suggest that PKS enzymes found in various lineages represent a conserved ancestral form derived from early eukaryotes.

PO.02-03
Molecular investigations on the toxic marine dinoflagellate *Alexandrium minutum*

Session: PO.02 - Genomics

I Jung[1], U John[1], G Glöckner[2], U Tillmann[1], B Krock[1], AD Cembella[1]

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Alexandrium minutum is a widely distributed HAB dinoflagellate that typically produces PSP neurotoxins (gonyautoxins 1,2,3,4), as well as poorly characterized allelochemicals. Within the EU-Project ESTTAL (Expressed Sequence Tags of Toxic Algae), we aim to identify candidate genes and processes putatively involved in toxicity and allelochemical interactions, and in growth regulation and stress responses. To obtain RNA samples linked with physiological status, batch cultures of *A. minutum* were grown under different environmental conditions, yielding differences in growth, PSP toxin production, and allelochemical potency. As a basis for gene expression analysis, we generated a normalized cDNA library, from which about 10,000 expressed sequence tags (ESTs) were generated. Annotation of these data yielded first insights into the genome of *A. minutum*. Based on the EST library, we designed an oligonucleotide microarray, which will be used to screen for differences in gene expression among the differently treated *A. minutum* cultures. Here we present the results of the EST library analysis, the design of the microarray, and physiological data of cultures grown for gene expression analysis.

PO.06-24
Dynamic modelling of cyanobacterial blooms in lakes using ECO Lab

Session: PO.06 - Population dynamics

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Cyanobacterial blooms are well known to produce toxins that pose a health threat to humans. Therefore management of bloom situations are important issues to managers of bathing waters and drinking water reservoirs worldwide. An operational approach for management would be to model the blooms; thus being able to predict mass occurrences and evaluate various measures to reduce the bloom intensity and frequency. In the present study, a dynamic model has been established for a small Danish lake using the model tool ECO Lab. The objective was to identify the most important parameters that must be taken into account when setting up such models. The model describes the dynamics of the most important algal groups including cyanobacteria, using a dynamic description of the hydrodynamics of the lakes, weather conditions and nutrient loading. The model is calibrated against monitoring data. A first attempt was made to include predictions of the toxicity in various compartments: intracellular, in water, in sediment.

PO.15-12
Development of a comprehensive method for monitoring harmful algae using real-time PCR assay

Session: PO.15 - Monitoring

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In Japan, several harmful algae form red tides and often cause mass mortality of cultured fish and shellfish. In order to minimize the damage to the fisheries industry, it is necessary to monitor the causative harmful algae rapidly, sensitively, and comprehensively. In this study, we developed a sensitive monitoring method by using real-time PCR (qPCR) assay against three dinoflagellates, namely *Cochlodinium polykrikoides*, *Heterocapsa circularisquama*, and *Karenia mikimotoi*, and two raphidophycean flagellates, namely *Chattonella* spp. and *Heterosigma akashiwo*. The assay contains an efficient DNA extraction protocol from these harmful algae, and therefore DNA extraction for qPCR is necessary only once for monitoring of the algae. Detection and quantification with qPCR, where the detection sensitivity was only 1 cell for all targeted species, were not affected by growth stages and the presence of non-targeted species. QPCR assay also labelled the harmful algae in environmental samples, where no targeted species was found by direct counting. Therefore, this comprehensive assay will be a powerful tool for precise prediction of the occurrence of harmful algae.

PO.08-08
Effects of cyanobacteria on copepod egg production in the Gulf of Finland, Baltic Sea

Session: PO.08 - Toxicology

MR Karjalainen, E Lindén, S Viitasalo, M Viitasalo

Finnish Institute of Marine Research, HELSINKI, Finland

Cyanobacterial blooms occur in the Baltic Sea every summer. Copepod peak abundance overlaps with the cyanobacteria mass occurrences. Copepods, in turn, are the main

food source for planktivorous fish like the Baltic herring and sprat. Therefore the effect of cyanobacteria on copepod production can have an effect on fish nutrition as well. However, little information exists about copepod production rates in a natural phytoplankton community containing cyanobacteria.
The copepod (*Acartia* spp.) egg production capacity and faecal pellet production was studied experimentally during 2-week field studies in the Gulf of Finland, Baltic Sea in summer 2004 and 2005. The experiments were conducted onboard R/V Aranda, using different size fractions of natural phytoplankton bloom communities as food suspensions for copepods. Egg production rates were very low even in the control treatment, suggesting that copepods were food-limited in the field. The almost complete lack of pellet production in cyanobacteria treatments indicates that copepod feeding rates were low. Even though cyanobacterial blooms host a variety of heterotrophic organisms, which can be favourable food items for copepods, apparently they were not consumed by copepods when cyanobacteria were present. These findings suggest that cyanobacteria affect zooplankton feeding behaviour.

PO.01-06
Development of a real-time PCR-based nucleic acid test for the detection of *Dinophysis* species in Irish waters

Session: PO.01 - Genetics

SM Kavanagh

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Diarrheic shellfish toxin (DST) producing *Dinophysis* species occur in Irish coastal waters throughout the year, with cell numbers peaking during the summer months. The majority of closures of Irish mussel-farms are attributed to *Dinophysis* blooms. Routine monitoring of toxic phytoplankton from Irish coastal waters, carried out by the Marine Institute, currently relies on microscopic identification of target species and biochemical analysis of shellfish tissue. Nucleic acid tests based on fluorescent in situ hybridisation probes (FISH) and PCR technologies have been shown to be effective for monitoring toxic phytoplankton species. We are currently developing real-time PCR based tests to detect *D. acuta* and *D. acuminata* in Irish waters, for use in the phytoplankton monitoring programme. Single-cell PCR was performed to amplify the D1-D2 region of the large ribosomal sub-unit (LSU) from indigenous *Dinophysis* species. The resulting sequence information for *D. acuta* and *D. acuminata* was aligned with all available LSU sequence information for *Dinophysis* species from GenBank. PCR primers and FRET hybridisation probes specific for *D. acuta* and *D. acuminata* were designed for real-time PCR tests on the LightCyclerTM. These tests are currently being optimised on Irish water samples.

PO.05-28
Gymnodimine toxins in Tunesian shellfish

Session: PO.05 - Toxin analysis

Riadh Kharrat

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The causative agent of toxicity in shellfish from a localized area on the Tunesian coast has been isolated by high-performance liquid chromatography (HPLC), following bioassay–guided fractionation of a dichloromethane extract of shellfish. The identity and the purity of the isolated compounds were confirmed by liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS). According to its RF, its molecular mass and its fragmentation patterns the toxins were identified as a mixture of gymnodimine A and gymnodimine B at a concentration of 885 and 80 $.10^{-6}$ mol /kg of shellfish, respectively. In contrast to gymnodimine B, gymnodimine A exhibited high neurotoxicity in mice and was lethal following inoculation by peripheral and intracerebroventricular administration.

PO.05-02

Development of a highly sensitive determination method for cylindrospermopsin using LC/ESI-MS

Session: PO.05 - Toxin analysis

Sachiko Kikuchi

Tohoku University, SENDAI, Japan

Cylindrospermopsin (CYN) is a hepatotoxic alkaloid produced by several freshwater cyanobacteria including *Cylindrospermopsis raciborskii*. CYN-producing cyanobacteria are abundant in surface waters used as drinking water reservoirs throughout the world. The toxicity of CYN and its presence have prompted the World Health Organization guideline value for drinking water quality of 1.0 µg CYN/L. When the guideline value is determined, a highly sensitive determination method is imperative. In this study, a highly sensitive determination method was developed, using LC/ESI-MS, and HEPES (2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethane-sulfonic acid) as the internal standard. In the LC/ESI-MS, the retention times of CYN and HEPES were 12.41 and 14.21 min, and were observed at m/z 414.00 for CYN and 237.00 for HEPES, respectively. CYN was determined from peak area ratios in the selected ion monitor (SIM) mode of m/z 414.00/237.00. Linearity of this method was observed in the range of 0.05-25 µmol/L (0.1-52 ng/5 µl injected volume). The quantification limit (QOL) at a signal-to-noise (S/N) ratio of 10 was 158 pg. The method is useful for determination of CYN in environmental and/or drinking waters.

PO.10-14

Ecological niche of a marine red tide ciliate *Myrionecta rubra* revisited: multi-modes of nutrition in a single species

Session: PO.10 - Ecophysiology & Autecology

HS Kim[1], G Myung[2], KG Chang[2], W Yih[2]

[1]Ministry of Marine Affairs and Fisheries, KUNSAN, South Korea
[2]Kunsan National University, KUNSAN, South Korea

Myrionecta rubra Jankowski 1976 (=*Mesodinium rubrum* Lohmann 1908) is a marine kleptoplastidic (=retaining plastids of donor cells) ciliate feeding on cryptophyte species including *Teleaulax* sp. As a functional primary producer *M. rubra* is very common and often causes recurrent red tides in diverse eutrophic marine environments. Recently, we observed active bacterivory by *M. rubra* MR-MAL01 strain that had been isolated from Gomso Bay, Korea. Thus, the nutrition of *M. rubra*, a single ciliate species, encompasses 3 different modes such as photosynthesis, bacterivory, and algivory. Among the metazoan predators of *M. rubra*, calanoid copepods, mysids, larvae of ctenophores and anchovies, and spats of bivalves were reported. Feeding on *M. rubra* by kleptoplastidic dinoflagellates, *Dinophysis* spp. was suggested to explain the cryptophyte origin of the *Dinophysis* plastids (Jansson 2004). Here, *M. rubra* is to serve as a donor of its kleptoplastid for the secondary kleptoplastidy by *Dinophysis* spp. To conclude, the traditional ecological niche assigned to *M. rubra* in marine pelagic environment needs to be rearranged because of the recently unveiling reality of the very complicated pelagic food web surrounding *M. rubra*.

PO.05-32
Paralytic shellfish poisoning (PSP) toxins in *Alexandrium catenella* and *A. tamarense* isolated from southern coastal and offshore waters of Korea

Session: PO.05 - Toxin analysis

CH Kim, YS Kim
Pukyong National University, BUSAN, South Korea

Thirty-two isolates of *Alexandrium catenella* and *A. tamarense* from the southern coastal and offshore waters and from the Yellow Sea in Korea were analyzed for PSP toxins. All contained toxins in the range of 0.04-46.38 fmol/cell. In seventeen coastal isolates, the major toxins were C2, GTX1,4 and neoSTX, and the minor toxins were C1 and GTX3. However, in fifteen offshore isolates, the major toxins were C2 and GTX4, and the minor toxins were C2, GTX1,3,5, and neoSTX. Among them, eight isolates contained GTX5, which was not detected in any coastal isolates. In addition, there was a clear difference in the proportion of carbamate to N-sulfocarbamoyl toxins between coastal and offshore isolates, which were 69:31 and 44:56, respectively. The cluster analysis based on toxin composition and concentration divided the Korean *Alexandrium* isolates into three distinct groups, which were clearly characterized by a higher proportion of C2, neoSTX, and GTX4, respectively.

PO.01-17
Isolation of preferentially expressed gene between different mating type cells in the dinoflagellate *Alexandrium tamarense*

Session: PO.01 - Genetics

A Kobiyama, K Koike, T Ogata
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Sexual reproduction is an important process in the life cycle of *Alexandrium tamarense*. However, nothing is known about the molecular mechanism during sexual reproduction although it is thought that discrimination at the molecular level between different mating type cells is important. In this study, subtractive PCR was used to isolate a gene, which is differentially expressed in the different mating type cells of *A. tamarense*. After three rounds of subtractive PCR using cDNAs synthesized from mRNA of different mating type cells, subtractive cDNA libraries were constructed. One gene, AT4-3, showed a strong mating type-specific signal as the results from cDNA membrane array and RNA

dot blot analysis of randomly selected library clones. The complete nucleotide sequence of AT4-3 was determined by RACE method. BLAST homology search showed no similarity to any known genes or proteins. The predicted amino acid sequence of AT4-3 has an N-terminal signal peptide for extracellular secretion, an N-linked glycosylation site and eight cysteine residues in half of the C-termini. Although the function of this gene is unknown, the results provide the first evidence of intracellular variation between the different mating-type cells in dinoflagellates.

PO.09-08

Occurrence of bacterial protein that reacts with specific antibody against saxitoxin

Session: PO.09 - Toxin synthesis and chemical structure of toxins

Massaki Kodama, Yoshinobu Takata, Shigeru Sato

Kitasato University, OFUNATO, Japan

Previously, we reported on the occurrence of a saxitoxin-producing bacterium isolated from *Alexandrium tamarense* cells, and we suggested that PSP toxins of the dinoflagellate originate from intracellular bacteria. However, the toxin productivity of the bacterium was too small to explain the toxin amount of the dinoflagellate. It suggests that PSP toxins are intermediates of some bacterial substance. Recently, we have found that conjugates of toxins and thiol compounds are formed by transformation of 11-O-sulfate derivatives of STX such as gonyautoxin 1-4 to STXs. These findings show that PSP toxins are more reactive substances than previously thought, and suggest the possible occurrence of a bacterial substance bound to PSP toxins. Using the reaction of PSP toxins with the thiol compound, the antigen in which STX is coupled with BSA was prepared for the specific antibody that shows affinity to all the toxin components examined. The western blot analysis of the bacterial protein extract using the antibody against STX showed the presence of bands. The hydrolyzate of the protein also reacted with the antibody. These results suggest the occurrence of proteins with PSP toxin(s) as constituent(s).

PO.04-01

Growth of harmful blue-green algae after viable gut passage in crucian and silver carp

Session: PO.04 – Food chains

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The role of fish-gut passage enhancing harmful blue-green algae productivity has recently been reported for a number of species residing in eutrophic lakes. The aim of present work was experimental study of growth of harmful blue-green algae after passage through intestinal tract of crucian carp (*Carassius auratus* Linn.) and silver carp (*Hypophthalmichthys molitrix* Val.). Growth of harmful algae passed through the intestine of crucian and silver carp from small Siberian reservoirs were compared with those of harmful algae taken directly from the reservoirs subjected to blue-green algal

blooms. The dominant phytoplankton species in the reservoirs, *Microcystis aeruginosa* Kütz. emend. Elenk., showed a significant increase of growth after the passage. However other dominant phytoplankton species in the reservoirs, *Anabaena flos-aquae* (Lyngb.) Breb. and *Aphanizomenon flos-aquae* (L.) Ralfs, were not stimulated by the gut passage. Our results demonstrated that when *M. aeruginosa* was dominant, the effects of crucian and silver carp on increase of summer blue-green algal bloom could be significant.

PO.15-01
Monitoring for harmful plankton blue-green algae in small Siberian reservoirs

Session: PO.15 - Monitoring

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Results of summer toxic bloom monitoring in small reservoirs of Krasnoyarsk region (Siberia, Russia) – Bugach, Bereshskoe, Vetluzhanka - were analyzed. Investigations were conducted during the period of open water in 2003-2005. Water blooms in these reservoirs were caused by harmful blue-green algae and had two peaks. The first peak (June-July) was dominated by mass development of *Aphanizomenon flos-aquae* (L.) Ralfs and *Anabaena flos-aquae* (Lyngb.) Breb. The second peak (August-September) – by *Microcystis aeruginosa* Kütz. emend. Elenk. The biomass of blue-green algae reached 200 mg/l during the period of bloom. The most important part of research was development of practical measures directed at the prevention of harmful blue-green algae mass development in these reservoirs. Complex 'top-down' and 'bottom-up' biomanipulation should be conducted for prevention of the water bloom. Levels of sediments and phosphorus in the water, the number of planktivorous fish need to be reduced, th hypolimnion aerated, and a coastal woody vegetation must be planted.

PO.13-56
Seasonal dynamics of harmful algae and their amino acids in two small Siberian reservoirs

Session: PO.13 - Regional events

AA Kolmakova, GS Kalachova, EA Ivanova

Institute of Biophysics of SB of RAS, KRASNOYARSK, Russia

A comparison of the dynamics of harmful algal biomass and total amino acid composition was made for two small Siberian reservoirs. The only factor that significantly changed the percentages of amino acids in water was the bloom of Cyanophyta in the 'blooming' water body. During the bloom of harmful Cyanophyta, the absolute and relative content of leucine-glutamine group increased, while the concentrations of other acids generally dropped. Before and after the bloom, no significant variation in the total amino acid composition was recorded. In the reservoir, where harmful Cyanophyta didn't dominate, no significant variations in amino acid composition were recorded during the season.

PO.10-02
ASP toxin composition of pennate diatoms and bacterial effect on the composition variation

Session: PO.10 - Ecophysiology & autecology

Yuichi Kotaki[1], N Lundholm[2], T Katayama[1], EF Furio[3], ML Romero[4], JR Relox[4], T Yasumoto[5], H Naoki[5], MY Hirose[5], TD Thanh[6], CV Thuoc[6], NTM Huyen[6], PT Thu[6], Y Takata[1], M Kodama[1], Y Fukuyo[7]

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Previously, we reported that Philippine strains of *Nitzschia navis-varingica* were separable into two types based on the toxin composition: DA-IB and IA-IB types. The former produced domoic acid (DA) with isodomoic acid B (IB), and the latter isodomoic acid A (IA) with IB. We further extended toxin analysis to many culture strains isolated in Japan, Vietnam, and Philippines. The IA-IB type was limited to only 13 strains from three areas of the northern Philippines and all other 183 strains belonged to the DA-IB type. When sub-strains were prepared in non-axenic conditions, they kept the same toxin type of the parent strain. However, when sub-strains were prepared in axenic conditions, one sub-strain changed itself from the DA-IB type to the IA-IB type. It returned to the original DA-IB type, when the culture medium was replaced with a cell-free but non-axenic medium of the parent culture, suggesting a bacterial role in controlling the toxin type. Toxin compositions of *Pseudo-nitzschia* strains isolated from Ofunato Bay, Japan, will be also presented.

PO.12-11
PLANKTON*NET a distributed online taxonomic database system – its benefits for harmful algal research

Session: PO.12 - Taxonomy and phylogeny
Presentation time: 16:40 - 18:00

A Kraberg[1], D Vaulot[2], DJ Patterson[3], A Ardelean[4], A Amorim[5], I Probert[6], J Young[7], T Moita[8], A Macario[1], KH Wiltshire[1]

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Many research projects globally are investigating harmful algae. These require accurate information on the taxonomy of different harmful species and their biogeography to target their research. PLANKTON*NET (e.g. http://www.awi.de/Plankton-Net) is a communal online database project with currently 6 European and 1 US partners. It aims to provide comprehensive information including images, taxonomic descriptions and matrix keys on marine and freshwater plankton. The content will serve the needs of many users, including the HAB community. PLANKTON*NET is conceived as an open-ended network of collaborating web sites (nodes) using micro*scope

software. The resources of all nodes will be linked so that content from all local sites, as well as external resources accessed through linkouts, can be accessed from each node. PLANKTON*NET will use taxonomically intelligent web services developed by the uBio Project (http://www.ubio.org) to organize information about organisms. The modular software will also be extended to contain environmental data. PLANKTON*NET already contains many records of harmful algae in collections from very different geographic areas and the coverage will increase as new partners make their data collections visible through PLANKTON*NET. By providing comprehensive, reliable up to date information it will present a valuable resource for research and monitoring studies on harmful algae.

PO.07-09

Effects of nutrient supply ratios and initial community composition on dinoflagellate bloom formation: mesocosm studies from the northern Baltic Sea

Session: PO.07 - Ecology and oceanography

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Since the early 1980s 'red-tides' caused by cold-water dinoflagellates have become a regular phenomenon in the coastal N Baltic Sea, where they increasingly replace the typical diatom-dominated phytoplankton spring bloom assemblage. Although this trend has generally been related to anthropogenic nutrient enrichment and changing climatic conditions, the mechanisms underlying the relationship have remained poorly understood. Using mesocosms, we studied the effects of variable nutrient additions and nutrient supply ratios on species composition and dominance patterns of natural spring phytoplankton communities from the coastal N Baltic Sea. The experiments which were repeated during 3 consecutive years showed that bloom formation and dominance of the dinoflagellate *Woloszynskia halophila* primarily depended on the size of the inoculum population and the relative abundance of co-occurring diatoms. Variations of N/P:Si ratios did not have any significant effect on phytoplankton development and composition. Addition of N and P as well as increased irradiance generally stimulated the growth of diatoms, but could not alter the outcome of competition between diatoms and dinoflagellates when the latter were initially dominant. Our results emphasize the importance of efficient recruitment strategies and initial conditions in dinoflagellate bloom formation.

PO.05-10

Yessotoxin profiles from cultures and planktonic field samples of the marine dinoflagellates *Protoceratium reticulatum* and *Gonyaulax spinifera*

Session: PO.05 - Toxin analysis

B Krock

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Yessotoxins are a large group of ladder-frame disulfated polyethers. Yessotoxin and an array of derivatives including glycosides can accumulate in suspension-feeding shellfish, leading to positive responses in the mouse bioassay for lipophilic marine biotoxins. Yessotoxins are globally distributed in coastal and shelf waters of diverse locations, including Japan, Norway, Chile, New Zealand, Italy and the North Sea. There are three known natural sources of yessotoxins – all are marine dinoflagellates belonging to the gonyaulacoid species *Protoceratium reticulatum*, *Lingoludinium polyedrum* and *Gonyaulax spinifera*. Yessotoxins were originally classed as among the diarrhetic shellfish poisoning (DSP) toxins, but they are now regarded as a distinct group as they do not induce diarrheagenic symptoms. We analyzed the yessotoxin composition of *P. reticulatum* isolates from the Benguela Current, South Africa and compared the profiles to cultures of *P.reticulatum* and *G. spinifera* from the North Sea off the east coast of Scotland by high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The data on the cultured isolates were then compared to the yessotoxin profiles found in natural phytoplankton assemblages from both locations.

PO.05-01

Direct selective separation of domoic acid by molecularly imprinted polymers

Session: PO.05 - Toxin analysis

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In the analysis of domoic acid (DA) in shellfish, phytoplankton and seawater as well as human samples such as blood and urine, several methods have been reported. However, the procedures of the methods are complicated. In order to simplify the procedures, selective separation of DA and its isomers from samples is the most effective. For the reason above, we developed a novel selective separation medium of DA using the molecular imprinting technique (MIT) that is the most effective method for selective molecular recognition. In this study, we used an advanced method of MIT, named 'fragment imprinting technique'. To design a template for DA, we focused on carboxylic acid groups in the molecule, and selected several carboxylic acid group-containing compounds. In the case of o-phthalic acid as a template, the selective recognition of DA was observed by high performance liquid chromatography (HPLC), while the medium prepared using m- or p-phthalic acid as the template recognized DA only to a small extent. Finally, we achieved direct selective separation of DA from shellfish-extracted sample by HPLC using o-phthalic acid imprinted polymer.

PO.08-16

Effects of microcystins on human polymorphonuclear leucocytes

Session: PO.08 - Toxicology

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Universidade de São Paulo, SAO PAULO, Brazil

Microcystins (MCs) are cyclic heptapeptides produced by cyanobacteria present in contaminated reservoirs. Reported toxic effects for microcystins are liver injury and tumour promotion. In this study, we evaluated the effects of two MCs, MC-LR and [Asp3]-MC-LR, on the human neutrophil cells (PMN). We observed that even at concentrations lower than that recommended by World Health Organization for chronic exposure (0.1 nM), MCs affect human PMN. Both MCs have chemotactic activity, induce the production of reactive oxygen species, and increase phagocytosis of *Candida albicans*. MC-LR also increased *C. albicans* killing. The effect of MCs on PMN provides support for a damage process mediated by PMN and oxidative stress, and may explain liver injury and tumour promotion associated with long-term MCs exposure.

PO.11-08

Allelopathic activity of *Alexandrium catenella* grown under N- or P- deficient conditions

Session: PO.11 - Allelopathy

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We demonstrate that *Alexandrium catenella*, a dinoflagellate species responsible of recurrent paralytic shellfish poisoning (PSP) blooms in Thau Lagoon has an allelopathic activity on co-ocurring phytoplankton species. *Alexandrium catenella* cell-free filtrate obtained in late exponential phase decreases significantly (Anova-test) the growth rate of target species. Because nutrients are usually present in low concentration *in situ*, we investigated the growth of the diatoms *Skeletonema costatum*, *Thalassiosira weissflogii*, and the dinoflagellates *Scripsiella trochoidea* and *Prorocentrum minimum* in the presence of the filtrate of *A. catenella* grown under N- or P- deficient conditions.
Results show that nutrient limitation of *A. catenella* cultures increases significantly its inhibitory effect on the growth of the tested microlagae. It also enhances the production of temporary cysts by the target microalgae.
These results suggest that *A. catenella* success in natural environments may be related to its capacity to produce allelochemicals against potential competitors.

PO.10-17

Pulsed phosphorus supply dynamics controlling the outcome of the competition between toxic *Alexandrium minutum* and non-toxic *Heterocapsa triquetra*

Session: PO.10 - Ecophysiology & autecology

C Labry, E Erard, A Chapelle, A Youenou, MP Crassous, J le Grand, B Lorgeoux

IFREMER, PLOUZANÉ, France

Since 1988 *Alexandrium minutum* has formed regular blooms in the Penze Estuary (northern Brittany, France) causing shellfish paralytic toxin contamination. This study aims to establish knowledge bases on the competition with a coexistent species, *Heterocapsa triquetra* and finally on the ecophysiology of *Alexandrium*. A preliminary study revealed that *Heterocapsa* always dominated in excess nutrients but phosphate limitation conditions followed by a phosphate supply enhanced the growth of *Alexandrium*. Different phosphate depletion times before a pulse were tested in mixed batch cultures. These experiments showed two contrasted periods. *Heterocapsa* grew preferentially for the first three days of depletion while *Alexandrium* growth was promoted after three days. This transition corresponded to two favourable physiological adaptations of *Alexandrium* cells: the use of stored intracellular phosphorus for later growth and a high capacity to increase its cell phosphate uptake rate. After three days, *Alexandrium* consumed the whole phosphate pulse, reducing *Heterocapsa* growth. These adaptations were confirmed in a P-limited semicontinuous culture experiment testing several phosphate supply frequencies (1, 2, 4, 6 days). *Alexandrium* always outgrew *Heterocapsa* and more quickly so for shorter intervals. The experiments revealed that *Alexandrium minutum* is a 'storage specialist' and *Heterocapsa triquetra* rather a 'velocity adapted' species.

PO.15-14

Solid phase adsorption toxin tracking (SPATT) from New Zealand to the Scottish Coast

Session: PO.15 - Monitoring

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At the XI International Conference on HABs it was proposed that SPATT was a simple and sensitive method for early warning of shellfish contamination. SPATT is founded on the observation that when low numbers of toxic algae are present in the water column significant amounts of toxins are dissolved in seawater. Experiments by researchers from New Zealand demonstrated a lag between detection of dissolved toxins adsorbed onto porous synthetic resin, phytoplankton peak cell densities and highest toxin concentrations in shellfish. Preliminary investigations at Loch Ewe (Scotland) used SPATT in the form of suspended SP-700 resin held within a mesh bag. Bags were suspended at 10 m and retrieved and replaced weekly when water samples for phytoplankton were also collected. The resin was extracted and analysed for lipophilic toxins using LC-MS. Okadaic acid and dinophysistoxins were detected at low concentrations in the absence of the causative *Dinophysis* spp. These concentrations increased prior to detection of *D. acuminata* and *D. acuta*. Yessotoxin was detected when the causative algae, *Protoceratium reticulatum*, was below the detection limit using light microscopy. We propose that SPATT is further developed for detection of domoic acid and saxitoxin using other available resins and computationally designed polymers.

PO.05-42

Evolution of DSP toxicity in a mussel-farming raft. Influence of bacterial faecal contamination and relative position in the raft

Session: PO.05 - Toxin analysis

J Lago, AG Cabado, JM Vieites

ANFACO-CECOPESCA, VIGO, Spain

Marine biotoxins are produced by dinoflagellates or other microalgae. Under optimal ecological conditions, toxic dinoflagellates proliferate, producing a bloom or red tide.

When a red tide occurs, filter-feeding bivalve molluscs accumulate the toxins produced by toxic dinoflagellates and become toxic for human consumption. Mussels are not affected by toxin accumulation, and when the bloom stops, they undergo a natural detoxification process.
The detoxification speed depends on many factors, not well understood so far. Among others, food availability has been proposed to play a positive role in shellfish detoxification. Food availability for mussels in a raft is affected by the their relative position, both in depth, because the algal population varies in the water column, and related to the water stream, because mussels downstream receive water which has been previously filtered by upstream individuals.
Our objective was to investigate if there is a relationship between DSP detoxification and the relative allocation on the raft. Our results suggest that upstream mussels lose DSP toxicity faster. Moreover, we studied the relationship between faecal contamination, coliforms and *Escherichia coli*, and DSP accumulation.

PO.13-87
On the genus *Alexandrium* (Dinoflagellata) in Vietnamese waters: - two new records of *A. satoanum* and *A. tamutum*

Session: PO.13 - Regional events

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Based on observations of cell morphology and thecal plate pattern, two dinoflagellate species, *Alexandrium satoanum* Yuki & Fukuyo 1992 and *A. tamutum* Montresor, Beran and John 2004 are reported here for the first time from Vietnamese waters. *Alexandrium satoanum* belongs to the subgenus Gessnerium and is characterized as follows: cells wider than long, ca. 40 µm wide and ca. 32 µm long, 1’ plate not connected to the APC and without a ventral pore. *Alexandrium tamutum* belongs to the subgenus Alexandrium and is characterized as follows: isodiametric cells, 25-30 µm, 1’ connected to the APC and with a ventral pore, 6' as wide as long, and sp. plate wider than long, cyst morphology unknown. With these records, 17 species of *Alexandrium* have now been reported from Vietnamese waters.

PO.08-03
DNA damage and apoptosis in CHO-K1 cells following treatment with cylindrospermopsin

Session: PO.08 - Toxicology

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The aim of our study was to examine the impact of cylindrospermopsin on DNA damage in CHO-K1 cells. The alkaline version of the comet assay, the micronucleus assay and flow cytometry were applied.
The source material for the production of cylindrospermopsin (CYN) was a culture of *Cylindrospermopsis raciborskii*. The concentration of CYN was

determined using LC/MS. CHO-K1 cells were treated with cylindrospermopsin at a dose of 0.5, 1 and 2 µg/ml for 24 hours. The level of DNA damage as well as the frequency of apoptosis were assessed by the alkaline comet assay. The Olive Tail Moment was calculated using the CASP software.
Micronucleus assay was used to correlate the results obtained with the comet assay with the cytogenetic results. The occurrence of early apoptosis was determined by the Annexin test and flow cytometry.
A missing correlation between the comet assay and the micronucleus assay results may suggest that cylindrospermopsin-induced DNA damage observed in the comet assay may be related to the early stages of apoptosis due to cytotoxic but not to genotoxic action of the compound.

This work was supported by the Polish Committee for Scientific Research No6PO5D01320

PO.03-03
Using beachfront restaurant sales in Southwest Florida to determine the localized impacts of HAB events

Session: PO.03 - Public health

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Extreme environmental events, such as red tide blooms, have been identified by owners and managers of waterfront-dependent businesses as a source of appreciable economic losses. For the restaurant sector in particular, these losses may be irrecoverable since products are perishable and consumption cannot be delayed entirely. In an effort to estimate the potential negative correlation between adverse weather events and short-run restaurant sales, proprietary sales data are combined with red tide and tropical storm data (incidence, intensity, wind speed, and wind direction). The observations are on a daily basis and cover three waterfront restaurants in Southwest Florida from January 1997 through September 2005. This presentation will illustrate the restaurant sales data, discuss the estimated models, and describe the relationship between the revenues and the various explanatory factors including red tides and tropical storms. In addition, the estimated impacts will be used to calculate the regional impacts associated with unforeseen environmental events that could aid in the development of a cost and benefit analysis of proposed expenditures on education, mitigation, research, and monitoring.

PO.10-40
Growth and toxicity of the dinoflagellate *Gambierdiscus toxicus* under nitrogen and phosphorus limitation

Session: PO.10 - Ecophysiology & autecology

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Caribbean strains (CCMP 1651 and 1655) of the toxic dinoflagellate

Gambierdiscus toxicus were grown in N- (nitrate, ammonium, free amino acids, urea, or putrescine) or P-limited (phosphate, ß-glycerophosphate, or nucleotides) xenic batch cultures. Growth rates ranged from 0.11- 0.18 d^{-1} except on urea (no growth). Cellular N and P pools were uncoupled from conditions in the water and cell division continued after the limiting nutrient was below detectable limits. N:P varied from 3-34 (1651) and 2-37 (strain 1655) with no relationship between cellular N:P and total toxicity. Strain 1655 was roughly five-fold more toxic than strain 1651. The N source had no effect on toxicity in either strain. The P source had no effect on toxicity in strain 1651, but strain 1655 cells grown on ß-glycerophosphate were more toxic. Stationary phase cells were more toxic than exponential phase cells in both strains under both N- and P-limitation. In P-limited semi-continuous cultures, the toxicity of strain 1655 decreased (43.7-79.3 fg C-Ctx1Eq $cell^{-1}$) as the growth rate increased (0.02-0.15 d^{-1}). Toxicity differences were a function of growth phase (batch culture) or growth rate (semi-continuous culture). Nutrient source and type of nutrient limitation having little to no effect.

PO.13-58
Involvement of cyanobacteria in the tropical ecotoxicological phenomenon of ciguatera fish poisoning

Session: PO.13 - Regional events

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During 2005, a cyanobacterial proliferation (*Hydrocoleum lyngbyaceum*) was found to induce a severe ciguatera outbreak in Lifou (Loyalty Islands, New Caledonia). Lipid-soluble extracts of cyanobacteria and giant clams were found to contain CTX-like compounds, as assessed by neuroblastoma cell bioassays and competitive membrane-binding assay using tritiated brevetoxin-3. According to the mouse bioassay, water-soluble extracts of both samples appear to contain the well-known alkaloid neurotoxins produced by cyanobacteria and responsible for Paralytic Shellfish Poisoning (PSP).
This is the first demonstration of simultaneous occurrence of PSP toxins and CTX-like toxins in extracts of marine cyanobacteria. The chemical identification of these toxins is currently underway by liquid chromatography coupled with mass spectrometry (LC/MS). Although long-term observations will be necessary to decipher the trends in dinoflagellate and cyanobacteria populations as well as the favourable environmental parameters, the new findings require that CFP risk assessment programs must now include monitoring of cyanobacteria besides the usual screening for dinoflagellates. In the perspective of global warming, such events would probably arise more frequently.

PO.11-02
Effect of bicarbonate addition on allelopathy in *Oscillatoria agardhii*

Session: PO.11 – Allelopathy

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Phytoplankton species compete with each other for limiting resources especially nutrients. This has led to the evolution of different strategies ensuring their competitive advantage over other species in resource utilization. An example of such a strategy is allelopathy – the release of organic compounds by plants into the medium, which affects competiting species. This study investigates the effect of bicarbonate-enriched spent media of non-toxic *O. agardhii* CYA 29 on growth and toxin concentration in *M. aeruginosa* PCC 7820.
Bioassays were carried out in BG 11 media to which were added either i) 15mM of bicarbonate ii) 0.1 % (v/v) spent medium of *O. agardhii* or iii) 15mM bicarbonate and 0.1 % (v/v) spent medium of *O. agardhii*. The cultures were incubated at 25 °C and sampled for biomass and toxin concentration.
A statistical analysis using Tukey's honestly significant difference test showed that after 3 weeks, biomass and total microcystin concentration were higher in cultures with spent media than those with bicarbonate and spent media. In contrast, microcystin concentration decreased six-fold in cultures with bicarbonate.
Conclusion: addition of bicarbonate and/or spent media of *O. agardhii* has a significant impact on growth and toxin production in *M. aeruginosa*.

PO.11-14
Epilithics biofilms: effect of allelopathic compound on structure and algal production

Session: PO.11 - Allelopathy

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Production of allelochemicals may be considered as a competitor- and predator-exclusion process. Epilithic biofilms are benthic microbial aggregates formed by an association of autotrophs and heterotrophs, prokaryotes as well as eukaryotes. With the relatively cohesive organization of microorganisms, these agglomerates are quite auspicious for allelopathic interactions. The aim of this study is to show the effects of the presence of an allechemical-producing strain on biofilm structure and production. The experimental set-up was based on artificial biofilms, grown in microcosms and composed of a limited number of algal species (cyanobacteria and diatoms, isolated from natural biofilms). Non-destructive techniques of biofilm investigations were used in order to elucidate both spatial structure and functioning (laser-scanning confocal microscopy, microelectrodes). To eliminate the effects of competition, extracts containing cyanobacterin, an allelopathic compound produced by the cyanobacteria *Scytonema hofmannii*, were added to the biofilms. In a more general context, epilithic biofilm is an example of complex integrated system whose dynamic functioning involves many signalisation and regulation networks. This study aim to understand how allelopathic interaction can modify biofilm functioning and structure.

PO.06-13
Effects of varying salinity and N:P ratios on the growth and toxicity of *Karenia brevis*

Session: PO.06 - Population dynamics

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The toxic dinoflagellate *Karenia brevis* forms extensive blooms in the Gulf of Mexico, releasing brevetoxins with implications for human health, mortalities of marine mammals and fishes. Historically, *K. brevis* was considered to form blooms above a salinity barrier of 24 PSU. Recently, blooms in low salinity waters were recorded in the Florida Panhandle and near the Mississippi River outflow. In this study *K. brevis* was cultured at salinities of 15-40 at 5 PSU intervals and N:P ratios (N:P = 16:1, 4:1 and 80:1), to measure the influence of salinity and nutrients on growth and toxicity. No growth of *K. brevis* occurred at 20 PSU or below, but good growth occurred at 25-40 PSU. Growth varied from 0.36 to 0.64 div day^{-1}. Highest growth rates occurred at 35 and lowest at 25 PSU. Salinity was a primary factor regulating growth, with nutrients as secondary factors. Using an ELISA assay the highest per cell concentration occurred in the 25 N-limited treatment with 100.0 and 40-Balanced with 99.2 pg toxin cell^{-1}. Similarly salinity was a primary factor regulating toxin production particularly at 25 and 40 PSU. Nutrient stress appears to stimulate toxin production, prompting further investigation as to the relationship between nutrients and toxins.

PO.10-19
Nutrient acquisition in the harmful dinoflagellate *Alexandrium tamarense* in response to different nitrogen supply

Session: PO.10 - Ecophysiology & autecology

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Nitrogen (N) is an essential variable controlling the bloom dynamics of dinoflagellates in marine environments. The ability to take up different species of N readily and simultaneously, and to switch N source rapidly is one of the mechanisms that may enhance the bloom potential for dinoflagellates. In the present study, dinoflagellate *Alexandrium tamarense* grown on a single N source was supplied with different forms of N, and two N species simultaneously to examine the cellular nutrient uptake kinetics under different supply mode. *A. tamarense* was able to take up N readily regardless of the previously supplied N source. Cells grown on either nitrate or urea were observed to take up ammonium rapidly. However, with ammonium-grown cells lower uptake rates of nitrate and urea were observed. *Alexandrium tamarense* demonstrated the ability to take up two N species simultaneously. When ammonium and nitrate (or urea) were supplied in equal concentrations, more ammonium was taken up and assimilated. The ability to take up different species of N rapidly and also simultaneously is one of the advantages that may allow *A. tamarense* to proliferate and form blooms in marine environments with complex N sources.

PO.01-20
The distribution of *Alexandrium* species in British coastal waters

Session: PO.01 - Genetics

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In 1968 the first instance of PSP toxicity in the UK was documented, linked to *Alexandrium tamarense*. *Alexandrium* species are a regular feature of the early summer phytoplankton assemblage in various parts of the country and PSTs are regularly recorded in shellfish. Over the years a range of studies on *Alexandrium* have been carried out in various parts of the country and further *Alexandrium* species (*A. minutum* and *A. ostenfeldii*) have been detected. The advent of molecular studies and the identification of two ribotypes for *Alexandrium tamarense* (North American and Western European) that occur in the UK, have further complicated this picture. The overlap in occurrence of these ribotypes is particularly interesting from an ecological standpoint and as part of the EU funded SEEDS project we are investigating the occurrence and mating capability of *Alexandrium tamarense* strains around the coast of the British Isles. In this presentation we will bring together all that is known (from disparate sources) of the occurrence of *Alexandrium* species in British coastal waters and highlight areas which merit more detailed investigation.

PO.08-17
Production of spirolides in single cells of *Alexandrium ostenfeldii* throughout the diurnal cycle

Session: PO.08 - Toxicology

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The production of toxic metabolites by dinoflagellates is known to be affected by both environmental and genetic factors. Changes in toxin content during the cell cycle have been observed in several toxic species including Alexandrium spp. In this study, a micro-column LC-MS system was used to quantify toxin concentration within a single cell over the diurnal cycle, allowing the accurate determination of changes in spirolide quota of single cells of AOSH1, an isolate of *Alexandrium ostenfeldii*. Digital image analysis was used to determine the volume of individual cells, which facilitated accurate calculation of des methyl-C concentration following analysis. In addition, changes in mean cell size in the culture were monitored using a Coulter Multisizer. A distinct advantage of this method was that the toxin profile and concentration could be determined for individual cells undergoing division. These methods allowed further examination of differences in the bio-volume of live and preserved (Lugol's) cells.

PO.10-25
Nitrogen uptake rates by successive dinoflagellate blooms in the East China Sea, 2005, and variation with nitrogen and phosphorus status

Session: PO.10 - Ecophysiology & autecology

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During late spring and early summer of 2005, large scale (>10,000 km^2) mixed dinoflagellate blooms developed in the coastal East China Sea, associated with massive fish kills in coastal aquaculture areas. Samples were collected from different stations along both north-south and west-east transects during 3 cruises of the Chinese Ecology and Oceanography of Harmful Algal Blooms (CEOHAB) Program, from April-June 2005, for assessing rates of N (NO_3^-, NH_4^+, urea, and glycine) uptake during these blooms. Nutrient uptake rates and preferences varied during the blooms, which were explained by the change of phytoplankton community, total nutrient availability, and its relative composition. Pre- and early-bloom rates showed high uptake of nitrate, but increasing dependence on reduced nitrogen sources as the blooms progressed. Later in the bloom, nitrogen uptake rates also increased with supplemental phosphorus enrichment, suggesting physiological phosphorus limitation.

PO.01-26
Genetic diversity studies on *Skeletonema* species (Bacillariophyta) in the coastal waters of southern China by SSU rDNA sequence analysis

Session: PO.01 - Genetics

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Small subunit (SSU) rDNA sequence analysis was applied to study genetic diversity of ten strains of *Skeletonema* collected from coastal waters of southern China. SSU gene fragments (approximately 1666 bp long) were cloned, sequenced and analyzed for comparison with morphological characters. Based on morphology, three species were identified: *S. subsalsum* (Cleve) Bethge (mainly from Shenzhen water), *S. marinoi* Sarno et Zingone (from Xiamen and Hongkong waters) and *S. dohrnii* Sarno et Kooistra (from Xiamen and Hongkong waters). All strains had polymorphic sites, with proportions ranging from 0.1% to 1.4%. Samples of *Skeletonema subsalsum* showed higher sequence diversity (from 1.3% to 1.4%) compared to the other strains. It was also found that *S. subsalsum* formed an individual lineage in NJ and UPGMA evolutionary trees. The strains of *S. marinoi* and *S. dohrnii* showed very low sequence divergence (ranging from 0.1% to 0.3%). Thus, molecular analyses using SSU rDNA were able to clarify the genetic diversity and taxonomic complexity of *Skeletonema*.
This work was supported by NSFC (40476055) and China 973 program (2001CB409701, 2005CB422305)

PO.13-81
Monitoring a bloom of *Pyrodinium bahamense* var. *compressum* occurring in El Salvador, Guatemala and Mexico (November 2005-March 2006)

Session: PO.13 - Regional events

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Water samples were analyzed by inverted microscope, and saxitoxin (STX) content of dead turtles was measured by HPLC methods. During the present bloom one person died and seven intoxications occurred. In addition, 206 deaths of turtles and numerous quantities of jellyfish were found on the Salvadorian beaches. Data analysis from Sea WiFS and Modis showed a positive temperature anomaly (1-5 °C) in November 2005 between Costa Rica and Southern Mexico lasting until March. Chlorophyll a data registered a positive anomaly as well (0.5-1.0 mg/m^3) following a similar path. The concentration of STX in turtles ranged from 27.9 - 627.8 µg STX eq/100 g, while on collected live jellyfish 17.3 - 21.4 µg STX eq/100 g were detected. Concentrations of STX in oysters collected in Mexico had 58 µg eq/100 g in December 2005 and up to 200.44 µg /100 g on February 2006. Microscope analyses revealed the presence of 48,900 cells ml^{-1} in December 2005 and a minimum of 15 cells l^{-1} in March 2006 in Salvadorian waters; while in southern Mexico maximum density was 12 cells l^{-1} on February 2006. Discussed also are ocean colour imagery collected during previous blooms in the same area and unpublished data obtained from Mexican coasts between December 1989 to February 2002.

PO.09-01
New gonyautoxin analogue isolated from the toxic dinoflagellate *Alexandrium minutum* (Dinophyceae)

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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Six clonal cultures of *Alexandrium minutum* established from Hai Phong, Northern Vietnam in October 2004 were examined for toxin production. The cultures contained predominantly GTX4. Other toxin congeners GTX1 - 3, NEO and dcSTX were also detected. Toxin content (Qt) varied among the strains and growth stages, and ranged from 3.0 to 12.5 fmol PST cell^{-1}. Interestingly, a peak eluted between GTX4 and GTX1 was consistently detected in all *A. minutum* strains with concentrations depending on the growth phase. The peak disappeared under non-oxidizing HPLC-FD condition but remained unchanged after treatment with 0.05 M ammonium phosphate/ 10 % mercaptoethanol or 0.1 N HCl hydrolysis. The peak was partially purified using Bio-Gel P2 after GTX1 - 4 were converted into STXs with ammonium phosphate /mercaptoethanol treatment. In LCMS, mass spectra of the peak showed predominant [M+H]+ ion at m/z 396. Ion scanning from 150 to 450 Da showed similar fragmentation to other GTX 1-4 with parental ion of [M+H]+ at m/z 396,and fragment ions [M-SO3]+ at m/z 316, and [M-SO4]+ at m/z 298.

The congener was identified as a deoxy GTX4-12ol lacking an oxygen atom at position C12. This is the first report of the congener.

PO.02-09
Is application of quantitative-PCR possible for measurements in toxic *Microcystis* populations?

Session: PO.02 - Genomics

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Six characteristic segments, mcyA~E, and the promoter of the microcystin synthetase gene cluster, designed in a Quantitative-PCR amplification were applied for quantitative measurements of toxic populations in environmental samples. Observations during the method-development experiments against 8 toxic and 4 non-toxic clones of *Microcystis* were: (i) the expected specific amplicons were found in all toxic clones but were absent or less abundant in non-toxic clones; (ii) all the toxic clones showed consistent Tm values of mcyD, implying this partial mcyD segment as the most conserved among the tested mcys; (iii) a linear correlation was obtained between the microscopically determined cell numbers and the PCR threshold cycles; (iv) cell concentration of the toxic *Microcystis* from Q-PCR measurement was not affected by addition of non-toxic populations; (v) gene copy number per cell was variable during the different growth stages and a maximal of 20 at the early logarithmic and stationary phase of batch cultures of toxic *Microcystis*. This Q-PCR can be applied in the environmental samples to as few as 800 toxic cells. The selection of suitable primer pairs for specific mcy segments is important to avoid misleading results.

PO.10-01
Effects of UVBR on different strains of the cyanobacterium *Nodularia spumigena* from the Baltic Sea

Session: PO.10 - Ecophysiology & autecology

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Nodularia spumigena is one of several toxin-producing cyanobacteria in the Baltic Sea. It produces the hepatoxin nodularin, a tumour promoter known to have killed wild and domestic animals. *Nodularia spumigena* blooms occur during late summer, a period with strong light, calm weather and stable water-column stratification. The tolerance to high light intensities of three different strains of *N. spumigena* was tested for 10 days in two different laboratory experiments. Cultures were kept in semi-continuous growth and exposed to UVBR and UVAR, up to 0.8 W/m^2 and 5 W/m^2, respectively. Variables measured: growth (light microscope), photosynthetic capacity (phyto-PAM, Walz), content and composition of photosynthetic pigments, phycobilin pigments and UV-absorbing compounds (HPLC). Despite some treatment effects, UVBR intensities up to 0.4 W/m^2 do not seem to have a negative impact on the three strains on day 10. However, when exposed to UVBR intensities up to 0.8 W/m$^{\mathbf{2}}$ all strains were to some extent negatively affected by a lower maximum quantum yield of photosynthesis but not growth. In conclusion, this toxic cyanobacterium appear to have the potential to outcompete less UVBR-tolerant taxa at increased levels of UVBR.

PO.13-35
Spatial and temporal analysis of PSP toxins in plankton and mussels along the Swedish West Coast

Session: PO.13 - Regional events

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Blooms of *Alexandrium tamarense*, *A. minutum* and *A. ostenfeldii*, known to produce PSP- toxins, occassionally occur in Swedish waters. During the last 10-15 years, sporadic testing of PST by the mouse bioassay in farmed blue mussels (*Mytilus edulis*) has shown that the regulation limit for these toxins is rarely exceeded. However, due to the prohibition to perform mouse bioassay in Sweden, very few data on the spatial and temporal distribution of PST in mussels exist. Further, there are no studies confirming the identity of the species responsible for PST production in Sweden. During the last years, HPLC analysis of PST has been established as a research tool in Sweden. This paper reports the initial results from a 2-year monitoring study, where plankton and mussels were sampled along the Swedish west coast using a spatial and temporal hierarchial sampling design. The results showed that PST levels above the limit for marketing were detected in more than half of the samples from May and June 2005. These findings indicate that PST is more common than previously believed and may constitute a serious threat to consumers of uncontrolled mussels.

PO.13-19
Blooms of *Alexandrium ostenfeldii* in a shallow archipelago area in Åland, SW Finland

Session: PO.13 - Regional events

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Summer blooms of the marine dinoflagellate *Alexandrium ostenfeldii* were observed in a shallow, densely vegetated archipelago channel in Föglö, Åland (SW Finland) in 2003-2005. In brown water with densities of about 1000 cells/ml, chlorophyll a values exceeded 10 microgram/l. The blooms were associated with strong bioluminescense, a phenomenon not previously reported from Åland. Thus, it is possible that *A. ostenfeldii* has reached Åland recently. The blooms were patchy and transported back and forth in the area. Phototactic behaviour and fast swimming of *A. ostenfeldii* in response to local water currents (partly caused by scheduled ship traffic in a nearby fairway) may have contributed to the bloom formation. No harmful effects were observed in the field, but toxicity is suspected in cultured material.

PO.12-12
Toward integrating molecular data into the process of recognizing new dinoflagellate species

Session: PO.12 - Taxonomy and phylogeny

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Dinoflagellate taxonomy is based on morphological criteria that are inherently more difficult to obtain than are corresponding DNA sequence data. This results in the rapid accumulation of sequence information indicating many more species exist than can be adequately described given the extant morphological data. Currently, no method exists for systematically categorizing these sequences into potential species groups for use in ecological studies or for taxonomic characterization. We examined whether genetic distances among ITS1/5.8S/ITS2 rDNA sequences could be used for this purpose. The analysis involved identifying sequences from 81 dinoflagellate species to determine if simple uncorrected genetic distances (p) above a certain level consistently correlate with known species boundaries. For a diverse assemblage of dinoflagellate species, the within species genetic distances between ITS1/5.8S/ITS2 copies (p=0.000-0.021 substitutions per site) were consistently less than those observed between species (p=0.042-0.580). Our results indicate that a between species uncorrected genetic difference >0.04 could be used to delineate most dinoflagellate species, including cryptic forms. Recently evolved species, however, may have ITS p values <0.04 and require more extensive morphological analysis, in combination with rRNA monophyly as a grouping criterion, to resolve. The overall significance of the correlation between morphology and molecular sequence data will be discussed.

PO.14-09
Inhibitory mechanism of acetone extract from *Eichhornia crassipes* root on *Prorocentrum donghaiense* Lu

Session: PO.14 - Mitigation

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The effects of *Eichhornia crassipes* roots on the growth of *Prorocentrum donghaiense* Lu were studied and the chemical basis of the inhibition examined. The effects of dry powder and acetone extracts of *E. crassipes* roots on *P. donghaiense* were observed. The components in acetone extract from *E. crassipes* were analysed by GC-MS and HPLC and the inhibitory activities of the main components in the extract were determinated. When *E. crassipes* roots dry powder was below 0.5 g L^{-1}, the growth of *P. donghaiense* was stimulated, whereas the growth was inhibited completely when the concentration were higher than 1.0 g L^{-1}. The growth of *P. donghaiense* was inhibited more than 50% by 0.019 g L^{-1} acetone extract of the roots. 1 mg L^{-1} of N-phenyl-2-naphthylamine was shown to have above 60% inhibitory effect on *P. donghaiense* after 6 days. 50 µL L^{-1} of linoleic acid resulted in 82% inhibition. GC-MS and HPLC showed that, in addition to N-phenyl-2-naphthylamine and linoleic acid, long chain fatty acids such as hexadecanoic acid, 9-hexadecenoic acid were present in the extract in relatively high amounts. The results showed that *E. crassipes* roots could inhibit the growth of *P.*

donghaiense, and that N-phenyl-2-naphthylamine and linoleic acid might play an important role in the inhibition.

PO.05-44
Production of monoclonal antibody and development of an enzyme-linked immunosorbent assay for the determination of okadaic acid in shellfish

Session: PO.05 - Monitoring

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An indirect competitive enzyme-linked immunosorbent assay for quantitation of okadaic acid(OA), a marine biotoxin associated red tides, was developed by preparation of a monoclonal antibody against OA using the cell-fusing method. OA-KLH was used as immunogen, and the spleen cells of immunized mice were fused with Sp2/O cells. Clones secreting specific monoclonal antibodies were screened by indirect ELISA using OA-OVA as coating ligand. After cloning, three hybridoma cell clones stably producing anti-OA monoclonal antibody were obtained. Detection of OA concentrations by indirect competitive ELISA was established. The detection limit of OA was 0.781ng/ml. The level of OA in shellfish from China was determined using the idc-ELISA method. A good correlation ($R=0.7963, P < 0.001$) was observed between idc-ELISA and HPLC-MS/MS.

PO.10-28
Response to small-scale turbulence by natural microphytoplankton assemblages along a natural *Alexandrium minutum* bloom event

Session: PO.10 - Ecophysiology & autecology

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Small-scale turbulence is an environmental factor that may directly affect different physiological processes of phytoplankton. Most available data have been generated in the laboratory using monospecific phytoplankton cultures. In the present work we used field samples of a bloom of dinoflagellates dominated by *Alexandrium minutum*, *Scrippsiella* spp. and *Prorocentrum micans*, in different phases of the succession, and we tested their sensitivity to experimentally generated small-scale turbulence.
Three experiments, each 15 days apart, were performed on the microplankton fraction (15-60 µm) of the natural water. The samples were distributed in 4-liter spherical flasks and maintained still or exposed to turbulence generated by an orbital shaker (turbulent dissipation rate epsilon~15 $cm^2 s^{-3}$) for ca. 10 days. The response was evaluated in terms of biomass yield, net growth rate and cyst abundance of the dominant species.
Diatoms, *A. minutum* and *Scrippsiella* spp. collapsed under still conditions while *P. micans* growth was not favoured by turbulence. Temporary cyst formation of *A. minutum* was

markedly reduced in the shaken treatments. The different degree of sensitivity to turbulence exhibited by each species appears to be linked to the initial physiological state of the community during the bloom.

PO.09-11
A new yessotoxin isomer from *Protoceratium reticulatum*

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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A new isomer of yessotoxin was isolated from extracts of New Zealand *Protoceratium reticulatum* cultures during large-scale purification of yessotoxin. Structural information was gained by LC-UV, LC-MS3 and NMR spectroscopy. The information obtained identifies the isomeric alteration is confined to the side chain substructure. The structural variation results in an increase in hydrophilicity of the molecule and a markedly earlier elution time compared to yessotoxin under reverse-phase chromatographic conditions. LC-UV and LC-MS3 data also confirmed the presence of other unknown yessotoxin analogues in the *P. reticulatum* extracts.

PO.08-11
Effects of cyanobacteria ingestion on *Daphnia magna* midgut epithelium and associated diverticula

Session: PO.08 - Toxicology

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The effects of ingested CYN (cylindrospermopsin)-producing cyanobacteria on the digestive tract of *D. magna* were investigated by light and electron microscopy. Cladoceran survival when feeding on cyanobacteria was also evaluated. We used as food three cyanobacteria differing in their ability to produce CYN: CYN-producing *C. raciborskii*, CYN-producing *A. ovalisporum* and CYN-non-producing *C. raciborskii*. In order to analyze cyanobacterial nutritional value, individuals were also exposed to a green alga (*Ankistrodesmus falcatus*). Other controls were given no food. The midgut from individuals fed with CYN-non-producing *C. raciborskii* revealed two distinct features: some individuals showed features like unfed individuals, whereas others showed features similar to those fed *A. falcatus*. In contrast, the midgut from individuals fed *A. ovalisporum* showed features resembling those of unfed individuals. In individuals of *D. magna* fed CYN-producing *C. raciborskii*, the midgut showed severe cell disorganization, suggesting another active metabolite. Our results show that cyanobacteria can be a toxic and inadequate food source for *D. magna*, due to low digestibility and to toxin content. The toxic effects observed were also illustrated by survival evaluation. Moreover, the CYN-producing *C. raciborskii* strain

used in this study may, in addition, produce another(s) secondary metabolite(s) responsible for the disruption of epithelial cell adhesion systems, but not yet identified.

PO.12-04
Evolutionary relationships between two winter-blooming photosynthetic dinoflagellates and heterotrophic *Pfiesteria*-like species

Session: PO.12 - Taxonomy and phylogeny

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Peridinium aciculiferum is a freshwater dinoflagellate normally found in north-temperate lakes. *Scrippsiella hangoei* is a marine-brackish dinoflagellate endemic from the Baltic Sea. Both morphospecies are important components of the winter phytoplankton community and generally bloom below the ice-cap at the end of the winter. The two morphospecies differ in habitat, general morphology and physiology. However, they share identical nuclear ribosomal DNA (SSU, ITS1-2, 5.8S and LSU) and very similar mitochondrial cytochrome b (COB) sequences, indicating a recent evolutionary divergence. AFLP fingerprinting shows that the two morphospecies are presently genetically isolated. The SSU and COB phylogenies indicate that both morphospecies are evolutionary related to estuarine species similar to *Pfiesteria*. Morphological data agree with phylogenies: the plate patterns of *P. aciculiferum* and *S. hangoei* are very similar to the *Pfiesteria*-like Shepard's-Crook species, and the three taxa appear as most closely related in the SSU phylogenies. Some *Pfiesteria* and *Pfiesteria*-like species are well known toxin producers. Interestingly, *P. aciculiferum* is one of the few freshwater dinoflagellates known to produce allelopathic substances, and there is some evidence that *S.hangoei* produce toxins. An interesting difference is that *Pfiesteria* and *Pfiesteria*-like species are heterotrophic, while *P. aciculiferum* and *S. hangoei* have chloroplasts. We are presently investigating the origin of their chloroplasts.

PO.08-22
Comparative pathogenicity of *Cochlodinium polykrikoides* from the York River, Virginia, USA and the Gulf of California

Session: PO.08 - Toxicology

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Cochlodinium polykrikoides is a globally distributed mixotrophic dinoflagellate that forms massive blooms worldwide. These blooms have been associated with fish kills in Japan, Korea, the Gulf of Mexico and the northwest coast of North America and *C. polykrikoides* is often considered a toxic alga, although the mechanisms of pathogenicity have not been clearly identified. Other regions including the east coast of North America also experience blooms of *C. polykrikoides*, although no association with fish kills has been reported. We conducted comparative larval fish bioassays (*Cyprinodon variegatus*) with bloom-derived cultures of *C. polykrikoides* collected from the York River,

Virginia and a clonal culture obtained from Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mexico. Larval fish exposed to whole-cell York River culture experienced 100% mortality in <8h while fish exposed to York River bloom lysate or whole cell and lysate fractions of the Gulf of California isolate experienced no mortalities in preliminary assays. Additional mortality assays, histological analysis of exposed fish and possible mechanisms of pathogenicity will be discussed.

PO.07-04
Ecological study of a *Karenia mikimotoi* bloom in the East China Sea in 2005

Session: PO.07 - Ecology and oceanography

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A huge bloom of *Karenia mikimotoi*, co-occurring with *Prorocentrum donghaiense*, has been recorded in the coast of Zhejiang Province, the East China Sea. The bloom covered an area of 15,000 km^2 and lasted about one month. A succession of species were recorded during the bloom, beginning with *Karenia mikimotoi* and *Prorocentrum donghaiense* and followed by *Noctiluca scintillans*. The population dynamics of *Karenia mikimotoi* was studied and the relationship between the bloom and environmental factors such as salinity, temperature, fronts, and nutrients was analyzed. Possible outbreak mechanisms will be discussed.

PO.07-06
Succession pattern of HAB species before large-scale blooms of dinoflagellates in the ECS in spring 2004/2005

Session: PO.07 - Ecology and oceanography

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Large-scale blooms of *Prorocentrum donghaiense* in May have been a recurrent phenomenon for the last decade in the ECS. During the first ten days of April 2004, the density of *P. donghaiense* exceeded 10,000 cells/L and it was the dominant species of phytoplankton in the subsurface layer. The highest concentration 100,000cells/L in some stations near the isobath of 50 m, where the abundant source of causative species was present for subsequent development of massive blooms. In the spring of 2005 the sitution was slightly different. Following a diatom bloom dominated by *Skeletonema costatum* and *Thalassiosira curviseriata* in late April, *Karenia mikimotoi* became dominant followed by *Scrippsiella trochoidea* and *P. donghaiense* in the subsurface layer leading in late May to the development and outbreak of the largest bloom of this ichthyotoxic species recorded in the ECS. The change in oceanographic and nutrient conditions in winter and

early spring of 2005 obviously influenced the succession pattern of phytoplankton, inducing the unusual proliferation of certain algae such as *K. mikimotoi*.

PO.01-12
Genetic diversity within Baltic Sea populations of nodularin-producing *Nodularia spumigena* and non-toxic *Nodularia harveyana*

Session: PO.01 - Genetics

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The genus *Nodularia* was recently divided into seven species. Four species (*Nodularia spumigena*, *N. baltica*, *N. litorea*, and *N. crassa*) are planktic with the capability to produce gas vesicles. Three species (*N. harveyana*, *N. sphaerocarpa*, and *N. willei*) lack gas vesicles and grow in benthic, periphytic, or soil habitats. Methods involving the whole genome and 16SrRNA sequences have indicated the close overall relatedness of *Nodularia* strains and also distinguished the nodularin-producing strains from the non-toxic ones [1].
Recently, *Nodularia spumigena* (Huebel 1988/306) and *Nodularia harveyana* (Huebel 1983/300) from the Baltic Sea were tested for production of nodularin, and only *N. spumigena* proved to be toxic. Therefore, the gene clusters of both species were analysed. Characteristic differences were observed. Southern analyses demonstrated that the cluster of *N. harveyana* lacks DNA sequences encoding the subunits NdaE/F.

[1] M.J. Laamanen, M.F. Gugger, J.M. Lehtimaki, K. Haukka, K. Sivonen. 2001. Appl. Environ. Microbiol. 67:4638-4647.

PO.13-92
Cyanobacteria blooms - a possible cause of mass mortality of Lesser Flamingos in Lake Manyara and Lake Big Momela, Tanzania

Session: PO.13 – Regional events

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Limnological studies were conducted in three alkaline lakes (Lake Big Momela, Manyara and Embagai) with the aim of investigating the cause of mass mortality of the Lesser Flamingos in Lake Manyara and Lake Big Momela during July–August 2004. High concentrations, up to 150 million filaments per liter of the potentially toxic planktonic cyanobacterium *Arthrospira fusiformis* were found in surface scum of Lake Big Momela where Lesser Flamingos were dying at a rate of between 15 and 50 individuals per day during the study period. Gut content analyses indicated that *A. fusiformis* was the main food item in moribund flamingos. Mouse bioassay suggested that the crude microalgal extract dominated by *A. fusiformis* was toxic with all mice close to death becoming lethargic, with loss of balance, uncoordinated movements, intermittent tremors, dyspnoea with gasping followed by respiratory arrest. This observation gives circumstantial evidence that A. fusiformis at such high concentrations was toxic to the

Lesser Flamingo in Lake Big Momela.

PO.08-12
35 times higher content of PTX-2 in *Dinophysis acuta* compared to DTX-1

Session: PO.08 - Toxicology

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The diarrhea-producing microalgae *D.acuminata* and *D.acuta* are common and known as pectenotoxin producers. In 2001, water samples was taken on the Swedish West Coast at three different depths and analysed for pectenotoxins with LC-MS. Countings of *D. acuminata* and *D. acuta* were performed for comparing toxicity contents in the cells. September samples from Koljörfjord contained 4000 cells/L of *D. acuta*, which caused contamination of 800 µg DTX-1/kilo mussel-meat in blue mussels (*Mytilus edulis*). The content of DTX-1 in the *D. acuta* population was approximately 700 ng DTX-1/100L seawater. The *D. acuta* population contained up to 25000 ng PTX-2/100L, an amount which is 35 times higher. PTX-2 is quickly converted into PTX-2 seco acid by the mussel tissue. The permitted level of toxin in mussel meat used for human consumption is 5000 µg /kg for PTX-2 and PTX-2 seco acid and 160 µg/kg for DTX-1. We should take in consideration that the permitted level for PTXs is 31,25 times higher and 35,7 times for PTX-2 production in *D.acuta*. More attention should be taken to determine if the permitted level of toxin content should be increased.

PO.07-05
Transport of potentially harmful species by density-driven coastal jets in the western English Channel

Session: PO.07 - Ecology and oceanography

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Strong bottom fronts to the west of Brittany and along the southern coast of England exist in the western English Channel in summer. Strong flows associated with these fronts were evidenced by satellite tracked drifters. In the central region, flows were weaker due to a combination of tide and wind effects.
Noticeably discoloured water in the western English Channel in late June/early July 2003 was due to a bloom of *Karenia mikimotoi*, cell densities reaching 3.8 million cells/l, with associated chlorophyll levels of up to 70 mg m^{-3}. Underway measurements and satellite imagery revealed the bloom to be located in the central area of the Channel between 3 and 5°W. Cell densities of *K. mikimotoi* were substantially reduced in late August when blooms of *Pseudo-nitzschia* and *Emiliania huxleyi* were observed. Multivariate statistical analyses were used to summarise the phytoplankton data from both cruises. Without doubt, the most important aspect of physical oceanography relevant to harmful events in the near-shelf/coastal

region studied was the presence of density-driven coastal jets over the bottom fronts. A transport pathway for *Karenia* populations, and other forms, associated with these flows from Ushant, across the English Channel and around the Celtic Sea is discussed.

PO.05-26
Within-day variations in response of the mouse bioassay for diarrhetic shellfish poisoning toxin (okadaic acid)

Session: PO.05 - Toxin analysis

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The mouse bioassay (MBA) for testing of marine biotoxins is still an important tool in food safety monitoring. During the symposium 'Marine and Freshwater Toxins Analysis' held in Baiona, Spain last year, we reported that following i.p. injection of PSP the mice showed a tendency to die more quickly when injected in the morning compared to those injected in the afternoon. Recently, we found the same phenomenon when okadaic acid (OA) was injected in mice. That is, mice injected with OA tended to die more quickly when injected in the morning than when injected in the afternoon. Further, the death time following injection of OA, was significantly different ($p<0.05$) between normally-fed mice and starved mice. The time of injection may therefore influence the decision for regulation. Following the dose of OA we used (4 µg per mouse), the mice died after less than 12 h. We are now regulating the toxin dose to a death time of approximately 20 h. Using the lower doses of OA, we plan to examine again the differences in death time between mice injected in the morning and in the afternoon.

PO.10-20
Carbon and nitrogen uptake kinetics of the harmful dinoflagellate *Alexandrium tamarense* in response to nitrogen supply mode

Session: PO.10 - Ecophysiology & autecology

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Anthropogenic enrichment in the form of nitrogen (N) is one of the stimuli for dinoflagellate blooms. N sources are not always supplied to the coastal ecosystem continuously, but in pulses. When phytoplankton is exposed to different N species or different N supply modes, physiological changes including uptake kinetics can be predicted to occur in the cells. In this study, the effects of N sources, concentrations and supply modes on the nutrient uptake and C:N ratio of *Alexandrium tamarense* were examined. The cellular C uptake exhibited different patterns among N sources and also between the two supply modes. This suggests that the C requirement for synthesis of organic products such as lipids might depend on the N utilized. The cellular N uptake was observed to display opposite trend among the two supply modes at >12µM-N. This suggests that the metabolic pathways and assimilation of N may differ among N sources. Therefore, the type of N supply to coastal environments may contribute to the bloom potential of dinoflagellates. Information on the nutrient dynamics of dinoflagellates may

assist in controlling blooms of toxic dinoflagellate and thus preventing a bloom from being fully developed.

PO.05-29
Evidence of yessotoxins in Alfacs Bay - toxic effect evaluation by cell-based assays and toxin profile determination by liquid chromatography

Session: PO.05 - Toxin analysis

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In July 2005, a bloom of the dinoflagellate *Protoceratium reticulatum* at cell concentration levels of 1600 cells/L was detected in Alfacs Bay, in conjunction with the presence of *Dinophysis sacculus*.
Positive DSP mouse bioassay measurements indicated the presence of diarrhetic toxins in mussels. Analytical procedures were optimised and applied to phytoplankton and to mussel extracts. Mussel samples were first extracted in methanol/H2O (80:20), further percolated through an octadecylsilica cartridge, derivatized using the dienophile fluorescence reagent, DMEQ-TAD, and subsequently analysed by liquid chromatography coupled to fluorescence detection (LC-FD). Concentration levels of yessotoxin in mussels about 0.3 to 1.0 µg/kg were detected during this event. Phytoplankton samples were extracted by sonication with methanol, followed by a clean-up step. Okadaic acid concentration levels in these samples were also studied to confirm the toxic effects recorded along the *Protoceratium*/*Dinophysis* bloom. Evaluation of cell-based toxicity of field samples by estimation of cell viability, IC_{50} and morphological effects was studied and compared to the analytical measurements. *Protoceratium reticulatum* was previously detected in phytoplankton samples from the Ebro Delta embayments in summer 2001, and concentration levels of yessotoxin and homoyessotoxin were then confirmed in mussel samples.

PO.13-45
The influence of *Pseudo-nitzschia australis* blooms in shellfish domoic acid accumulation on the Andalusian coast (southern Spain)

Session: PO.13 - Regional events

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Diatoms of the genus *Pseudo-nitzschia* have been detected at the Andalusian coast since the monitoring program began in 1996. Some species from this genus are harmful because of the production of the toxin domoic acid. During 2006, samples from the monitoring program were analysed with LM and EM, allowing identification of *Pseudo-nitzschia australis* as the most abundant *Pseudo-nitzschia* species. This event resulted in marked toxin accumulation in several bivalve molluscs from the Mediterranean coast.

PO.05-07
Testing of a passive adsorption device in the detection of DTXs under controlled conditions

Session: PO.05 - Toxin analysis

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Recently, McKenzie and his collaborators described an attractive technique to detect lipophilic toxins in the field. The principle is based on the passive adsorption of toxins onto porous synthetic resin held in small bags. For the purpose of using this technique for a decontamination trial, an experiment was set up in the laboratory with a *Prorocentrum lima* culture. *Prorcentrum lima* is a benthic species producing DTXs and cultivable in the laboratory. The cumulative adsorption was studied under three environmental conditions: in the raw culture, in the filtrate and in the lysate of the same culture. The results obtained from this preliminary experiment were as follows: the cumulative adsorption does not seem to be modified by the cell presence, its relationship with exposure time is linear over time and for the toxin levels studied, and the reproducibility is good in all cases.

PO.15-16
The Environmental Sample Processor (ESP): a robotic device for detecting microorganisms remotely using molecular probe technology

Session: PO.15 - Monitoring

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One of our primary research goals is to develop analytical techniques and instrumentation that allow us to use molecular probe technology to detect microorganisms, their genes and gene products remotely, in situ. Towards that end we have developed the ESP, http://www.mbari.org/microbial/ESP, a device designed to collect discrete water samples from the ocean subsurface, concentrate particulates, and automate application of ribosomal RNA (rRNA) targeted DNA probe arrays (see Greenfield *et al*.) and antibody-based diagnostics (see Doucette *et al*.). The ESP can also be used to archive samples for a variety of nucleic acid analyses, microscopy and other types of analytical procedures after the instrument is recovered. To date, 3 different classes of DNA probe arrays that target a variety of bacteria and archaea, harmful algae, and larval invertebrates have been applied in single field deployments in Monterey Bay, CA, lasting ~20 d. In concert with the probe arrays for detecting a suite of HAB species that include *Pseudo-nitzschia australis*, *P. multiseries* and *P. pseudodelicatissima*, we have successfully utilized a competitive ELISA technique for detecting domoic acid. In this presentation we will review the design and workings of the ESP, and our plans for future deployments, developments and technology transfer.

PO.08-25
Effect of emersion on diarrhetic shellfish toxins depuration from the blue mussel *Mytilus galloprovincialis*

Session: PO.08 - Toxicology

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The effect of emersion on the depuration of DSP toxins DTX2, okadaic acid and conjugated forms of these two compounds, in the mussels *Mytilus galloprovincialis*, was studied. Contaminated mussels were placed in tanks with running seawater and maintained for one week. On days 2 and 5 of the experiment a subgroup of mussels was kept out the water for one day, another subgroup for 12 h, and a third subgroup was maintained submerged. The observed depuration was slow for the three treatments. Emersion seems to have no relevant effect on DSP toxin depuration. Notwithstanding, the relative contribution of conjugated forms was lower in the two treatments that involved emersion than in the one in which mussels were always submerged and in the initial samples, probably because of increased esterase activity in the two former treatments. This likely hydrolytic activity produces an increase of the free forms of the toxins after one week of depuration in the mussels subjected to emersion.

PO.08-10
Do toxic *Alexandrium minutum* strains affect feeding and survival rates of the pelagic marine copepod *Euterpina acutifrons*?

Session: PO.08 - Toxicology

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Short-term feeding and survival experiments with three different toxic strains of *Alexandrium minutum* revealed that the copepod *Euterpina acutifrons* was able to consume them to a similar extent at which they fed on the non-toxic and similar-sized *Scrippsiella trochoidea*. Feeding showed a typical satiation response to increasing food concentrations. For a given food concentration, no significant differences between the ingestion rates of *E. acutifrons* on the different dinoflagellate strains were found, except for differences between AL1V and *S. trochoidea*. Concentrated extracts of copepod tissues fed on *A. minutum* revealed the dominance of N - sulfocarbomoyl toxins. Some gonyautoxins were also detected in copepods fed on AL1V. At the end of the feeding experiments, organisms were healthy. During the 288-h exposure time no differences were found between survival rates of *E. acutifrons* when fed on AL1V (55 %) or AL2V (70 %) nor when fed AMINAR1 (80 %) or S. T. (95 %), but significant differences were observed between copepods fed both AL1V or AL2V with respect to AMINAR1 or S. T. Our results suggest that *E. acutifrons* can consume *A. minutum* and act as PSP toxin vector through the food web during bloom events.

PO.05-22
Isolation of novel spirolides from the marine dinoflagellate *Alexandrium ostenfeldii*

Session: PO.05 - Toxin analysis

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Spirolides are macrocyclic compounds characterised by a tricyclic ether system and a seven-membered cyclic imine moiety. The marine dinoflagellate *Alexandrium ostenfeldii* is the only known proximal source of these biologically active compounds that evoke apparent neurotoxicological symptoms in mice. In recent investigations of a strain of *A. ostenfeldii* (AOSH2) originating from Ship Harbour in Atlantic Canada, we found several previously undescribed spirolides. Precursor scans of characteristic fragment-ions by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) revealed molecular ion masses that did not correspond to known structures and exhibited fragment ion spectra that differed from spirolides of equal molecular weight. An LC-MS/MS method was optimised for the baseline separation of the complex spirolide mixture. Since the unambiguous structural elucidation of these compounds requires nuclear magnetic resonance (NMR) spectroscopy, dinoflagellate batch cultures were harvested to generate sufficient spirolides (upper microgram range) and high purity components for spectroscopic analysis. Low pressure column chromatography and solid phase extraction (SPE) techniques were employed to remove major matrix compounds from the raw cell extracts. These combined methods provide a feasible scheme for the production of high purity spirolides for structural elucidation.

PO.15-04
Status of potentially harmful algae in the Chesapeake Bay estuarine system

Session: PO.15 - Monitoring

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Chesapeake Bay is the largest estuary in United States. Two monitoring programs started in 1985 and 1998 have provided data regarding phytoplankton populations for Virginia tributaries and regions in Chesapeake Bay. These programs have resulted to date in the identification of 1454 taxa within these waters with 34 potentially toxic (2.3%) species. These include common bloom producers: *Akashiwo sanguinea*, *Cochlodinium polykrikoides*, and *Prorocentrum minimum*, plus non-toxic *Ceratium furca*, *Heterocapsa triquetra*, *Heterocapsa rotundata*, *Scrippsiella trochoidea*. Also, *Dinophysis acuminata* blooms in 2002 reached highs of 236,000 cells/l, with okadaic acid present. Long-term trend analysis has indicated significant (p=0.01) increasing trends in abundance and biomass of cyanobacteria in Virginia tidal river sections. For example, in 2004, *Microcystis aeruginosa* Potomac River blooms lasted 3 months at salinities up to 7.5 ppt. Although no re-occurring toxic events within this estuary have occurred, there are potentially toxic flora, which under specific environmental conditions, may

through increased development influence the health status and water quality within this system.
Supported by Virginia Dept. Environmental Quality, Virginia Dept. Health, and USEPA.

PO.08-15
Antimicrobial and cytotoxic assessment of marine cyanobacterial extracts

Session: PO.08 - Toxicology

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Marine cyanobacterial strains isolated from Portuguese rocky shores and adapted to large-scale laboratory culture were screened for biological activities. Seventeen strains belonging to the genera *Cyanobacterium*, *Oscillatoria*, *Synechocystis* and *Synechococcus* were tested for antifungal and antibacterial activity and ten of these strains were screened for cytotoxic activity against primary rat hepatocytes and HL-60 cells. Extracts of different polarities were tested. No inhibitory effects were found against the fungi *Candida albicans* and a wide range of Gram-negative bacteria. Nine cyanobacterial strains were found to have antibiotic activity against two Gram-positive bacteria, *Clavibacter michiganensis* subsp. *insidiosum* and *Cellulomonas uda*. Slight apoptotic effects were observed in primary rat hepatocytes when exposed to aqueous extracts but no significant apoptotic effects were registered when cells were exposed to organic extracts. A high percentage of apoptotic cells were observed for HL-60 cells when treated with the cyanobacterial organic extracts. Our data demonstrated that marine cyanobacteria extracts cause inhibition of Gram-positive bacteria and induce apoptosis in eukaryotic cells. The different activity in different extracts suggests different compounds with different polarities. Cyanobacterial strains of the genera *Synechocystis* and *Synechococcus* proved to be a potential source of bioactive compounds.

PO.13-86
The genus *Ostreopsis* in the recreational waters along the Catalan Coast and Balearic Islands (NW Mediterranean Sea)

Session: PO.13 - Regional events

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Two *Ostreopsis* species have been detected in the Mediterranean Sea, *Ostreopsis* cf. *siamensis* and *O. ovata*. Both are toxic (palytoxin analogues) and live loosely attached to macroalgae, although they have the availability to detach and swim in the water column. Respiratory problems have been recently related with these organisms in some Mediterranean coastal areas. In August 2004, 200 people were affected by respiratory difficulties in the central part of the Catalan coast. Studies on the distribution of these epiphytic dinoflagellates are scarce and it is an urgent task to be done due to its potential danger. During the tourist season, an extensive monitoring associated with bathing beaches was performed in two Mediterranean coastal regions exploited for recreational use.

Sampling was performed in 240 beaches along the Catalan coast (CSIC_Agència Catalana de l'Aigua) and 80 beaches along the Balearic Islands (CSIC_Conselleria del Govern Balear). Results revealed the widespread distribution of the genus *Ostreopsis* in the bathing waters of these two recreational coastal areas. Concentration of the organism around 200 cells l^{-1} is a common situation in the water column and in several occasions concentrations as high as 10^4 cells l^{-1} have been detected.

PO.01-22
Testing the hypothesis of temperate Asia origin of *Alexandrium catenella* in Thau Lagoon (NW Mediterranean) using microsatellite markers

Session: PO.01 - Genetics

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A massive bloom of *Alexandrium catenella* associated with paralytic shellfish poisoning first occurred in Thau Lagoon (French Mediterranean) in 1998. Since then, *A. catenella* has been repeatedly blooming in this lagoon. More recently, *A. catenella* blooms also occurred for the first time in several areas in the NW Mediterranean (Spain, Italy) where this species seems to be expanding. On the basis of genetic analyses of *A. catenella* strains isolated from the NW Mediterranean, using rDNA markers, it was suggested that this Mediterranean population could have been introduced from temperate Asia (e.g. Japan) through ballast waters. In order to test this hypothesis, we carried out a genetic analysis of French Mediterranean and Japanese strains using microsatellite markers (MS). MS sequences are highly polymorphic markers, which are nowadays widely used to study population genetics in terrestrial and aquatic macroorganisms. This approach has been recently used to investigate genetic diversity in phytoplankton. MS have been developed in *A. catenella* (Nagai *et al*., Molecular Ecology Notes 6:120-122, 2006). Using these markers, we will present the comparative analysis of *A.catenella* strains isolated from Thau Lagoon (from blooms and resting cysts) and from Japanese waters. The data are discussed with respect to analyses based on rDNA data.

PO.11-06
Inhibitory effects of diatoms on the growth of the dinoflagellate *Akashiwo sanguinea*

Session: PO.11 - Allelopathy

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Results of phytoplankton high-frequency studies over four years in Hakozaki Harbour, Hakata Bay, Japan, showed that the bacillariophytes *Skeletonema costatum* and species of the genus *Chaetoceros* are dominant in spring and summer, whereas *Asterionella japonica* is dominant in the fall. On the other hand, the density of *Akashiwo sanguinea* began to increase during early fall, when the diatoms began to decline. *Akashiwo sanguinea* occasionally formed blooms during late fall when all diatoms had disappeared. Thus, inhibitory effects on growth of *A. sanguinea* by these diatoms were examined under laboratory conditions. The growth of *A. sanguinea* was strongly inhibited in bi-algal cultures with *S. costatum* and *Chaetoceros didymium*, and slightly inhibited by *A. japonica*. Furthermore, the growth of *A. sanguinea* was significantly lower in filtrates (enriched with nutrient matter) containing medum on which *S. costatum* and *C. didymium* had grown thickly than in fresh medium. These results suggest that *A. sanguinea* was inhibited by allelopathy and cell contact with *S. costatum* and *Chaetoceros* sp. blooms in spring and summer. The growth rate of *A. sanguinea* increased in the fall, due to the absence of diatoms and disappearance of inhibitory conditions.

PO.13-88
Red tide due to the dinoflagellate *Karenia mikimotoi* occurred in Hiroshima Bay in 2002

Session: PO.13 - Regional events

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A large-scale red tide due to harmful dinoflagellate *Karenia mikimotoi* occurred in Hiroshima Bay, western part of Seto Inland Sea, Japan in 2002. Hydrographic and biological investigations were conducted at a monitoring station from the initial outbreak to cessation of the red tide. Visible blooms (>10^6 cells/L) occurred 6-22 July during the monitoring station. Maximum cell density was 1.35×10^7 cells/L at the monitoring station and 2.74×10^8 cells/L in a harbour located near the station. Water temperature and salinity during the outbreaks of the red tide ranged from 23.5 to 26.1 °C and 27.5 to 31.3 psu, respectively. Finfish and oyster aquaculture held in the bay were devastated by the red tide. Total fisheries damage to farmed finfish was 950,000 US$. The average mortality of the Pacific oyster *Crassostrea gigas* and the mussel *Mytilus galloprovincialis* were 46% and 65%, respectively. Massive kills of shellfish were probably caused from a detrimental effect of *K. mikimotoi* because the death of shellfish had occurred before the lowest values of of anoxic water (2.0 mg/L in bottom) had developed.

PO.01-14
A molecular approach to identify *Pseudo-nitzschia* species in natural samples

Session: PO.01 - Genetics

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During recent years, a number of new *Pseudo-nitzschia* species have been described (e.g. Lundholm *et al*. 2003) that are genetically distinct, but difficult to distinguish using light microscopy and in some cases even with electron microscopy. Therefore molecular methods must be developed to identify the various species for the purposes of both monitoring programmes and ecological studies at sea. A PCR-based method was employed to assess the seasonal distribution of *Pseudo-nitzschia* species during 2004 within the Gulf of Naples. A genus-specific primer set was derived from *Pseudo-nitzschia* LSU sequences and used for PCR on environmental DNA samples. Clone libraries were constructed from the DNA fragments obtained at six different dates, chosen among samples with maximum abundances and diversity of *Pseudo-nitzschia* species. Sequence analysis revealed 13 LSU types, ten of which correspond to known species such as *P. galaxiae* and *P. delicatissima* and three seem to be novel genotypes warranting further investigation. The composition of the clone libraries differed among the dates, reflecting seasonal succession among the *Pseudo-nitzschia* species as detected by LM identifications. The potential applications of this method are discussed as a means to support identification of *Pseudo-nitzschia* species in natural samples.

PO.06-19
Dynamics of *Prorocentrum lima* on mussel ropes and the implications for economic impact and site management

Session: PO.06 - Population dynamics

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Mussels harvested in May (2001) from a site in Newfoundland were found to contain DSP toxins and were rejected from European Union (EU) markets. The cost of the product and more importantly the cost of shipping were a significant economic loss to the producer. The Canadian Food Inspection Agency (CFIA) now routinely tests for DSP toxins in product destined for the EU in addition to the toxins that it had traditionally tested. The probable source of the DSP toxin in the mussel product was determined to be *Prorocentrum lima* growing epiphytically on the mussel ropes. This study examines the seasonality and spatial distribution of these dinoflagellates within the aquaculture site and in relation to the other epiphytic species found on the mussel ropes and collectors. Epiphyte samples were collected over a two- year period from mussel socks and collector lines and examined microscopically to determine the distribution of harmful algal species. The implications for site management and the subsequent economic impact on the producer are discussed.

PO.05-14
A fast and sensitive multi-analyte UPLC-MS/MS method for the detection of DSP and other lipophilic marine biotoxins in shellfish

Session: PO.05 - Toxin analysis

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The high sensitivity and selectivity of LC-MS/MS have proven to be reliable and effective in many areas of analysis where validated quantitation and confirmation are required. Recent advances in the technologies involved show great potential for the technique in the field of biotoxin research, where the detection of shellfish toxins is becoming increasingly reliant on instrumental methods.
The diversity of structures and chemistries exhibited by these compounds, the extreme complexity of the matrices and the requirement for a fast and efficient analysis have

given rise to considerable analytical challenges. The high resolution offered by Ultra-Performance LC helps to minimise co-elution of analytes and matrix and in turn reduces suppression effects in the mass spectrometer, increasing overall sensitivity. The latest generation of tandem-quadrupole mass spectrometers use novel ion optics and high performance electronics, enabling fast switching between MRM transitions and polarity switching, to allow for more analytes in a single run.
Presented here is a method developed for the analysis of 23 marine biotoxins from six distinct compound classes. Secondary, confirmatory transitions and calibration curves for the most important compounds are included and an overall cycle time of 6 minutes is achieved with LoDs from 0.17 to 43pg on-column.

PO.13-54
Blooms of *Pyrodinium bahamense* var. *compressa* along the Central American Pacific coast and south of México

Session: PO.13 - Regional events

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The first toxic episode of *Pyrodinium bahamense* in America occurred in Guatemala (July 1987), when 26 people died after consumption of clams. In Costa Rica and Tehuantepec Gulf (México) the first bloom was in October 1989, resulting in 99 sick people and 3 fatalities. The toxin level was 811 µg STXeq $100g^{-1}$. Subsequently (1995-1996), this species was registered again in México, reaching the coast of Michoacán, and causing death of marine organisms at a toxin level of 6,337 µg STXeq $100g^{-1}$. In Costa Rica a subsequent bloom took place 10 years later (1999-2001) covering the entire Pacific coast, and both morphotypes var. *compressa* and var. *bahamense* occurred. In Acapulco, México in 2001 the toxin level reported was 7,309 µg STXeq $100g^{-1}$. Meanwhile in Chiapas, resting cysts were registered in the water column from January 2001 and the first vegetative cells in March 2001. We observed exponential growth until the formation of the bloom six months later. Cysts were also observed in the water column when the bloom advanced. The last registration was in November-December 2005 in the Gulf of Papagayo (Costa Rica) with densities of $350x10^6$ cells l^{-1}. The same event was registered in Chiapas (México) from December to March 2006, reaching cell densities of 950 cells $l^{-1}$and a toxin level of 200 µg STXeq $100g^{-1}$.

PO.06-04
Dinoflagellate blooms and paralytic shellfish poisoning producers in Uruguayan waters, in relation to environmental conditions

Session: PO.06 - Population dynamics

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Toxic dinoflagellate blooms have been a recurrent phenomenon in Uruguayan waters since 1991. The objective of the present study was to analyze the relationship between

the PSP- producing species *Gymnodinium catenatum* and *Alexandrium tamarense* and several environmental factors in the period 1991-2004. The study zone included three Uruguayan coastal locations along a 140-km stretch: Piriápolis, Punta del Este and Arachania. The relation between weekly abundance of the two species, water temperature, salinity, temperature anomalies, seawater temperature (SST 3.4 Index) from NOAA, and Río de la Plata was analyzed.
The *A. tamarense* blooms higher than 10,000 cells/l occurred between 11 and 14 ºC, 17.3 and 32.2 psu, without strong incidence of temperature anomalies, and a Rio de la Plata flow between 17,000 and 23,000 m^3/s. *Gymnodinium catenatum* blooms occurred between 21.8 and 24 ºC, generally under positive anomalies of sea surface temperature, with salinities from 18.4 to 32 psu, under higher Rio de la Plata flow discharge between 18,000 and 34,000 m3/s.

PO.15-23
California Program for Regional Enhanced Monitoring of PhycoToxins (Cal-PReEMPT)

Session: PO.15 - Monitoring

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California's expansive coastline is threatened by blooms of a variety of harmful algal genera, including *Pseudo-nitzschia* and *Alexandrium*. Efficient and cost-effective new methods for species and toxin detection have been developed, as have remote sensing capabilities for bloom tracking. Although these technologies are available, a constraint to adoption of them by the California Department of Health Service (CDHS) is the lack of funds for ground-truthing them, a necessary step before full adoption and incorporation into the state's monitoring effort. To bridge the gulf between availability of new tools and integration of those into monitoring efforts, NOAA, through its Monitoring and Event Response Program for Harmful Algal Blooms (MERHAB), is providing funding to perform necessary validation of new tools for incorporation of them into the CDHS monitoring program. We have established pilot project sites where new technologies are incorporated into an intensive monitoring program. Our approach is to shift much of the monitoring effort to the field, where field technicians pre-screen samples for toxins and toxin-producing species, thus ensuring early warning of impending blooms while avoiding un-necessary and expensive lab-based sample testing. This presentation will provide an overview of our MERHAB-funded program, detailing our progress and accomplishments to date.

PO.12-09
Molecular phylogeny and ultrastructural studies of the periflagellar area of some benthic species of *Prorocentrum* (Dinophyceae)

Session: PO.12 - Taxonomy and Phylogeny

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The taxonomy of the genus *Prorocentrum* is currently in a state of flux. McLachlan *et al.* (1997) suggested reinstatement of the generic name *Exuviella* for some species presentl included in *Prorocentrum*. To further explore the taxonomy of '*Prorocentrum'* species we sequenced the nuclear-encoded large subunit (LSU) ribosomal DNA gene of some *Prorocentrum* species isolated mainly from Malaysia. Molecular phylogenetic analyses revealed a few lineages which could be divided into several groups supported by morphological characters such as the presence of trichocysts, ornamentation of the periflagellar area, ornamentation of valve surface and type of toxin. Additionally, the periflagellar area of two *Prorocentrum* species, viz. *P. emarginatum* and *P. concavum*, was studied by serial sectioning in the TEM to examine whether this character is a useful taxonomic character for inferring relationship at the species level. The platelets were labelled according to Taylor's scheme. The results showed that the platelets of the two *Prorocentrum* species were uniform in number, arrangement and ornamentation such as flanges and collars extending from the platelets. We conclude that additional gene sequence data are needed before the taxonomy of *Prorocentrum* can be finally settled with any confidence.

PO.10-15

Interaction effects of high irradiances and nutrient concentrations on the cyanobacterium *Nodularia spumigena* from the Baltic Sea

Session: PO.10 - Ecophysiology & Autecology

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Nodularia spumigena is one of the dominant species during the cyanobacterial blooms in the Baltic Sea. The blooms occur during late summer, a period with increased irradiances, low DIN:DIP and stable water-column stratification. It has been observed that increasing irradiance has a stimulating rather than an inhibitory effect on *N. spumigena*. Because of its ability for nitrogen fixation, a low DIN:DIP would not be expected to negatively affect *N. spumigena*. In this study we tested the interaction effect of high irradiance (PAR and UVBR+UVAR) and different DIN:DIP on a strain of *N. spumigena*. The laboratory experimental period was 17 days and the cultures were kept in semi-continuous growth and exposed to PAR and PAR+UVBR+UVAR. Variables measured: nutrient concentrations (PO_4^-, NO_3^-) growth (light microscope), photosynthetic capacity (phyto-PAM, Walz), content and composition of photosynthetic pigments (HPLC), phycobilin pigments (spectrophotometer), UV-absorbing compounds (HPLC), and nodularin (HPLC). The experiment is currently running and the results will be presented at the conference.

PO.06-14
The return of *Gymnodinium catenatum* after 10 years: bloom initiation and transport off the Portuguese coast

Session: PO.06 - Population dynamics

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The last bloom of *Gymnodinium catenatum* on the western Iberian coast was reported in 1995 although some cells were detected in the following years. Previous studies on the species dynamics suggest that bloom initiation occurred in Lisbon Bay in a retention area related to the Cape Roca upwelling plume. During 2005, in this Bay, *G. catenatum* was observed up by the monitoring programme from mid-July. In August/September, a survey carried out on the area showed that *G. catenatum* was distributed (>500 cells/L) along a strong thermal front, resulting from the interaction of the colder upwelled waters of Cape Roca plume and oceanic waters. Satellite-derived sea level anomaly maps revealed that during the cruise, and for at least three weeks before, the off-shelf surface circulation was dominated by the presence of two counter rotating mesoscale eddies, responsible for a strong northeastward flow of warm oceanic waters into the survey area. During autumn, the monitoring programme allowed the following of a northward shift of the population related to a poleward surface flow. On 29 November, *G. catenatum* reached the maximum concentration (43x$10^{\mathbf{3}}$ cells/L) on the Aveiro coast and lasted in northern Portuguese waters until January 2006.

PO.05-06
First evidence of DTX2 in France: detection by LC-ESI-MS2 during 2004-2005 south Brittany phytoplankton blooms

Session: PO.05 - Toxin analysis

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Our study relates to phytoplankton samples collected during one year on the southern Brittany coast (05/10/04-07/18/05). For each sample, we worked at the same time on taxonomic and toxin profiles (lipophilic phycotoxins). To ensure that all samples were treated identically for optimum reproducibility, all phytoplankton extracts were quickly purified on Solid Phase Extraction (SPE), with a robotic station (ASPECXli, Gilson). The simultaneous identification and quantification of toxin profiles were achieved by using a liquid chromatography–tandem mass spectrometry (LC–MS/MS) with electrospray ionisation (+) and monitoring of daughter ions in multiple reaction modes. The results obtained in the taxonomic analysis showed that *Dinophysis acuminata* was the predominant species (75%). The toxin analysis of the samples by LC-ESI-MS/MS shows that okadaic acid (AO) is the principal lipophilic toxin. Howeve,r 02/11/04 a monospecific sample containing 75% of *Dinophysis acuta*, was an exception to the uniformity of the profiles observed during this year. The LC-MS2 analysis of this plankton net tow shows the rare presence of DTX2 (dinophysistoxin-2). For this sample

(42 600 cells of *Dinophysis* spp/L), [AO] was 25,4 pg/cell, while for *Dinophysis acuta*, the [DTX2] was 25 pg of equivalent AO/cell.

PO.13-64
A post-tsunami study on the diversity of dinoflagellates in the coastal area of Phang-nga Province, Thailand

Session: PO.13 - Regional events

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An assessment of tsunami damage on the mangrove and coastal ecosystem of Nam Khem Village, Phang-nga province, southern Thailand was carried out in October 2005. The area was damaged by the tsunami event in December 2004. The water intrusion into mangrove creeks and the destruction of mangrove trees by the over-washed wave caused serious problems for both the ecosystem diversity and function. Thus, a study on the diversity and distribution of phytoplankton and benthic microalgae was conducted to assess the long-term impact of the tsunami. Duplicate water samples were collected from subsurface and bottom depths at 7 stations located along the water channel between the main land and Kho-kao Island and in the mangrove creeks. A total of 15 genera of dinoflagellates were found in the area with *Protoperidinium*, *Ceratium* and *Prorocentrum* as the most abundant genera. Dinoflagellate cysts were also observed. A high density of *Prorocentrum*, more than 200 cells/l, was recorded in the mid-channel and the adjacent mangrove creek where the salinity was higher than 32 psu. The characteristics and the distribution of this potentially toxic genus are discussed.

PO.01-07
Molecular characterization and morphological variability of seven strains of the dinoflagellate *Prorocentrum minimum*

Session: PO.01 - Genetics

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The class Dinophyceae has been the subject of several molecular studies that have clarified the phylogenetic position of dinoflagellates with respect to other protists and have helped to establish new genera. However, within the same dinoflagellate species, morphological features can sometimes vary in response to changing environmental conditions or physiological states. *Prorocentrum minimum* has been a subject of interest to taxonomists because of its highly variable cellular morphology. This has given rise to a debate about whether the different shapes represent separate species or only one variable taxon. The morphological diversity of seven strains of *P. minimum* from different geographical areas was investigated using a range of cell characters, and intraindividual genetic diversity was assessed by sequencing the internal transcribed spacer regions (ITS1 – ITS2). Three strains originated from the Adriatic Sea, two from the Baltic Sea, one from the Gulf of Finland and one from Chesapeake Bay (USA). All strains were kept under controlled conditions, at their original salinities. From the morphological and

phylogenetic analyses, it was concluded that size and shape variations are indicative of different morphotypes of the same species.

PO.16-10
Life cycle transformations in HAB species: *Pseudo-nitzschia* in the Gulf of Naples

Session: PO.16 – Life cycles

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The EU project SEED (http://www.icm.csic.es/bio/projects/seed/) aims at improving and extending our knowledge of the shifts between the different life stages in HAB species and at identifying the environmental and physiological factors that regulate those transitions. The two domoic acid-producing diatoms *Pseudo-nitzschia galaxiae* and *P. multistriata* regularly bloom in the Gulf of Naples (Mediterranean Sea). The former species consists of three genetically distinct populations with different seasonal timing of their bloom, whereas the latter species generally blooms in late summer-autumn, with a secondary peak in early winter. The temporal dynamics of their cell size spectrum may provide a hint for the occurrence of sexual reproduction. Do the different species have distinct environmental windows for optimal growth? Does a bloom represent the occasion in which sexual reproduction occurs? Where are the species when they are not seen in plankton? We provide preliminary data gained from laboratory experiments and in situ observations of *P. galaxiae* and *P. multistriata* in the Gulf of Naples.

PO.08-13
Lipid, fatty acid and sterol composition of 8 species of Kareniaceae: chemotaxonomy and putative lipid phycotoxins

Session: PO.08 - Toxicology

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Lipid class, fatty acid and sterol composition of 8 species of ichthyotoxic marine gymnodinioid dinoflagellates (*Karenia*, *Karlodinium*,*Takayama*) was examined. The common dinoflagellate polyunsaturated fatty acids (PUFA), octadecapentaenoic acid (OPA 18:5ω3) and docosahexaenoic acid (DHA 22:6ω3), were present in all species in varying amounts (14-35% and 8-23%, respectively). The very long-chain PUFA (VLC-PUFA) 28:7ω6 and 28:8ω3 were present at low levels (<1%) and the ratio of these fatty acids may be a useful chemotaxonomic marker at the species level. The typical dinoflagellate sterol dinosterol was absent from all species tested. A predominance of the 4-methyl and 4-desmethyl Δ8(14) sterols in all dinoflagellate species included 23-methyl-27-norergosta-8(14),22-dien-3ß-ol (*Karenia papilionacea*, 59-66%), 27-nor-(24S)-4a-methyl-5a-ergosta-8(14),22-dien-3ß-ol, NED, (*Takayama tasmanica* 84%, *T. helix* 71%, *Karenia brevis* 45%, *Karlodinium* KDSB01 40%, *Karenia mikimotoi* 38%) and (24S)-4a-methyl-5a-ergosta-8(14), 22-dien-3ß-ol, ED, (*K. mikimotoi* 48%, *K. umbella* 59%, *Karlodinium*

veneficum 71-83%). The relative levels of the PUFA OPA and DHA, coupled with the potential inhibitory action of Δ8(14) sterols, and the potential by many of these species to produce reactive oxygen species, may provide insight into the ichthyotoxicity of these bloom-forming dinoflagellates.

PO.15-32

Utilization of volunteers to monitor harmful algal blooms in the southeastern coast of the United States

Session: PO.15 - Monitoring

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The Southeast Phytoplankton Monitoring Network (SEPMN) was established by the National Oceanic & Atmospheric Administration (NOAA) as an outreach program to unite volunteers and scientists in monitoring harmful algal blooms. Currently, there are 60 volunteer groups that monitor 72 sites along the southeast Atlantic coast from northern Outer Banks, NC to Jacksonville, FL.
The Program is based on a volunteer network of schools, community groups, and parks/recreational facilities. Volunteers are instructed on algal identification and sample on a weekly/biweekly basis, reporting their data to researchers at the Marine Biotoxins Program. Results from volunteer groups enable researchers to identify problem areas to isolate for further study. A volunteer based monitoring network enables researchers to maintain and monitor an extended survey area throughout the year.
Since 2001, SEPMN has provided opportunities for teachers, students, and community members to participate in real scientific research, helping to expand knowledge about phytoplankton and the roles these organisms play in our daily lives. For more information about SEPMN, visit the website: http://www.chbr.noaa.gov/PMN/.

PO.12-02

Species of the genus *Pseudo-nitzschia* Peragallo (Bacillariophyceae) in Greek coastal waters

Session: PO.12 - Taxonomy and phylogeny

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Records of species belonging to the potentially toxic (Amnesic Shellfish Poisoning) diatom genus *Pseudo-nitzschia* in Greek coastal waters are limited. This study presents detailed information about the occurrence and morphological variability of *Pseudo-nitzschia* species in Greek coastal waters. A taxonomic survey was carried out at the main harvesting and shellfish growing areas 2004-2006. Species identification was based on light (LM) and electron (SEM and TEM) microscopy of field samples (preserved and live material) and unialgal cultures. Eight species/taxa were identified; *P. fraudulenta*, *P. multiseries*, *P. multistriata*, *P. pungens* and four species within the *P.pseudodelicatissima*/*cuspidata* complex: *P. caciantha*, *P. calliantha*, *P. pseudodelicatissima*, and a morphospecies that closely resembles what is described as *P. caciantha* (*Pseudo-nitzschia* cf. *caciantha*). Morphometric data are given and the observed

morphological variability of the species within *P. pseudo-delicatissima/cuspidata* complex is discussed. The spatial distribution of the various species is also outlined.

PO.09-02
Enhancement of gymnodimine production in automated culture of *Karenia selliformis*

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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Cawthron, NELSON, New Zealand

Gymnodimine has recently attracted interest because of its pharmacologically interesting properties mainly residing in the spiro-cyclic imine ring, which is thought to activate L-type calcium channels of brain receptors. In order to investigate its pharmacology, a requirement for the production of gymnodimine is the guarantee of a reliable and enhanced supply of the compound. We have constructed a photoreactor (15 L capacity) allowing improved light access (190 µmol/m^2/s) operated in a 12h light:12h dark cycle, with gentle stirring and aeration of culture. The unit also has an inlet for carbon dioxide injection, and capability of pH and turbidometric measurements. We have operated the reactor on a semi-automatic basis with pH poised at 8.5. Growth kinetics and gymnodimine production by *K. selliformis* (growth yield maximum, 46510 cells/ml; doubling time, 8.6 days; gymnodimine, 0.5 µg/ml) were substantially improved compared to standard (12 L) batch culture (growth yield max. 41,510 cells/ml; doubling time, 17.3 days; gymnodimine, 0.16 µg/ml) with no pH amendment. As a consequence a fully automated culture system has been developed. We describe parameters for growth and gymnodimine production by *K.selliformis* with respect to fixed culture pH in this system.

PO.10-27
Comparison of growth rate and efficiency of the Texas brown tide alga *Aureoumbra lagunensis* when grown on DON and DIN

Session: PO.10 - Ecophysiology & autecology

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The nuisance Texas brown tide (TBT) alga, *Aureoumbra lagunensis*, reached bloom densities once again beginning in the spring of 2005. Although the original Texas brown tide bloom persisted 1990-1997, there is only limited information on the growth or nutritional characteristics of the causative organism. We investigated the effect that organic nitrogen availability and reduced photosynthetically available radiation (PAR) have on the growth of TBT. Such parameters seem critical for blooms of the morphologically and genetically similar east coast brown tide species *Aureococcus anophagefferens*. We tested the ability of TBT to utilize DIN (NO_2^- or NH_4^+) and DON (urea or glutamic acid) as the sole source of nitrogen under 8 light intensities ranging from limiting to saturating irradiances using a light gradient table. The experiments demonstrated highest

TBT growth on NH_4^+ (1.0 div day^{-1}), followed by urea (0.8 div day^{-1}), glutamate (0.54 div day^{-1}), and NO_2^- (0.48 div day^{-1}). Growth rate vs. irradiance curves revealed that the tested forms of organic nitrogen do not enhance growth of the TBT at any irradiance above that observed for NH_4^+ grown TBT. We hypothesize that the TBT may utilize ambient DON to sustain growth at lower rates when DIN becomes limiting.

PO.10-21

Nitrate and phosphate uptake kinetics of the dinoflagellate *Alexandrium tamarense* in relation to N:P supply ratios

Session: PO.10 - Ecophysiology & autecology

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Nitrogen (N) such as nitrate, and phosphate (P) are primary nutrients in coastal ecosystems. Their concentrations vary so dramatically that phytoplankton is bound to be exposed to variable N:P supply ratios. The present study investigated nitrate and phosphate uptake parameters of *A. tamarense* in relation to N:P supply ratios in a semi-continuous experiment. *Alexandrium tamarense* was grown at five different N:P supply ratios (N:P = 4, 8, 16, 32 and 64) by keeping phosphate concentration at 1.56 µM-P with variable nitrate concentrations. Increasing the N:P supply ratios induced a decrease in cellular nitrate uptake rates. However, cellular phosphate uptake rate showed a maximum value at the N:P supply ratio 16. The cellular C:N ratio decreased with increasing N:P supply ratio, and reached minimum value at high N:P supply ratios. This suggests that the dinoflagellate may respond differently to nitrate or phosphate due to N:P supply ratio. Therefore, the N:P supply ratio in coastal environments should be taken into consideration when assessing the nutrients utilized by dinoflagellates such as *Alexandrium* species.

PO.13-72

Brevetoxin contamination is common in fish from the eastern Gulf of Mexico

Session: PO.13 - Regional events

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Brevetoxins are potent neurotoxins produced by a few species of harmful algae, most notably *Karenia brevis*. These toxins are highly ichthyotoxic, leading to massive fish kills during red tide events. Fish can be exposed to brevetoxins by absorption of dissolved toxin through the gills, or by ingestion of *K. brevis* cells or toxic prey such as shellfish, seagrass or zooplankton. Although very susceptible to soluble brevetoxins, a series of experimental exposures of fish to contaminated prey revealed that ichthyotoxicity is modulated by the route of exposure which allows for fish survival and toxin accumulation in tissues by dietary transfer.
In light of these results and given the involvement of brevetoxin-contaminated fish in a 2004 bottlenose dolphin mortality[1], live fish throughout the Gulf Coast of Florida (n>500, more than 69 distinct species) were collected and analyzed. Our results indicate that

brevetoxin accumulation in fish is much more common than initially expected. More than 70% of the fish analyzed were found to contain detectable levels of brevetoxins in their tissues. Prevalence, toxin levels and distribution in tissues from fish at all positions in the food-web are discussed with regards to human and natural resources health.

[1]Flewelling, Naar *et al*. Nature 2005 435:755-756

PO.15-11
Rapid detection of toxic *Alexandrium* species by Loop-mediated isothermal amplification, a new DNA amplification method

Session: PO.15 – Monitoring

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In order to detect toxic *Alexandrium* species, we designed and evaluated a novel DNA amplification method using Loop-mediated isothermal amplification (LAMP). This method synthesizes a large amount of DNA with high specificity, sensitivity, and rapidity under isothermal conditions at 60-65 °C, and it is unnecessary to use expensive equipments (thermal cycler and Q-PCR system). The method employs a DNA polymerase with strand displacement activity and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. This method can be monitored the target DNA in real-time by increase in turbidity due to an abundance of the by-product, pyrophosphate, and also detected the DNA in the presence of fluorescent intercalating dye with the naked eye. The primers targeting the D1/D2 region of the large-subunit rDNA were designed in each species. LAMP was carried out in a reaction mixture containing Bst DNA polymerase and its appended buffer, dNTPs, and betaines to screen for specificity, rapidity and simplicity i.e. primer combination and DNA extraction, etc. The LAMP method is a simple method which can detect the DNA of target species from a single cell in natural samples within 20-25 min with high specificity (<60 min in total (n=16)).

PO.13-12
Epiphytic dinoflagellates from the Brazilian coastline

Session: PO.13 - Regional events

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Reef flats of the tropical and sub-tropical Brazilian coastline can be regarded as one of the preferred habitat types for epiphytic dinoflagellates associated with macroalgae. However, little is known about this local assemblage. Epiphytic dinoflagellates are the source of toxins that cause ciguatera, a human poisoning that results from consuming toxic finfish from coral reefs. This study aimed to investigate the epiphytic dinoflagellate species composition and abundance on macroalgae from Muro Alto (8° 27'S, 34° 59'W) and Maracaípe (8° 32'S, 35° 00'W) on the northeast, and Arraial do Cabo (22° 59'S, 42° 00') on the southeast Brazilian coast. Macroalgal samples were collected from tidal pools in algal reefs and from rocky shores. They were placed in plastic bags and a known volume of filtered seawater was added. Bags were shaken for 1 min to dislodge epiphytic microalgae. The suspension was preserved with neutral lugol iodine for microscopic identification and enumeration. Epiphytic microalgae were quantified by sedimentation in a settling chamber and examined under an inverted epifluorescence microscope using calcofluor staining. Species composition and abundance are reported. Species of *Prorocentrum* and *Ostreopsis* were observed, indicating the potential risk of toxin accumulation through the food web.

PO.04-02
Short-term feeding response of the mussel *Mytilus chilensis* exposed to diets containing the toxic dinoflagellate *Alexandrium catenella*

Session: PO.04 – Food chains

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Frequent blooms of *Alexandrium catenella* in southern Chile encouraged undertaking of the present study, which uses the mussel *Mytilus chilensis* as a model for evaluating the feeding response to diets containing PSP produced by *A. catenella*. Short-term feeding responses were measured using four diets containing different proportions of *Alexandrium catenella*. Four specimens were exposed to diets containing *A. catenella* and three controls were fed with a diet free of *A. catenella*. Diets containing the highest percentages of *A. catenella* significantly affected clearance rate during the initial hours. After this period *M. chilensis* demonstrated an ability to acclimate to toxin-containing diets, approaching similar values to the control mussels. The relative insensitivity of *M. chilensis* to PSP resulted in the rapid normalization of its feeding behaviour, allowing it to accumulate the paralytic toxin within a short period of time. This capacity made *Mytilus chilensis* a good indicator species for detection of PSP events potentially dangerous to human health. The capacity for acclimation of *M. chilensis* may be an adaptive property within the natural population of origin, which was affected by the toxic bloom of *A. catenella* during 2002.

(We are grateful for financial support from: Grant FONDECYT 1030340 and DID-UACH).

PO.13-10
HABs and hurricanes in Florida

Session: PO.13 - Regional events

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The 2004 and 2005 hurricane season provided two unique opportunities to evaluate the effects of post-hurricane nutrient enrichment of the West Florida Shelf (WFS) and HAB initiation and maintenance. Multidisciplinary research cruises began approximately 10 days after both Hurricanes Charley and Wilma, the former occurred during a period without a HAB event and the latter occurred during a HAB event. In both cases, nutrient loading on the WFS was up to an order of magnitude greater than pre-hurricane, wet season conditions and much greater than dry season conditions. Nutrient bioassays taken at the mouths of bays and rivers during each cruise indicated that nitrogen was the most limiting nutrient in these locations, although P co-limitation was also implicated in some regions and light was the primary limiting factor for phytoplankton growth in about half the locations. Comparisons of limiting nutrients in similar bioassays within offshore and inshore blooms during non-hurricane conditions provide supporting evidence of nutrient needs in *Karenia brevis* blooms. The passage of Hurricanes Katrina and Rita ameliorated a HAB related hypoxia/anoxia event on the WFS.

PO.12-13
Pseudanabaena cf. *moniliformis*, a new toxic cyanobacterium from Vietnam

Session: PO.12 - Taxonomy and phylogeny

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A freshwater cyanobacterium from Huong River (Hue, Vietnam) has been isolated into clonal culture (strain HOs24). Based on the general morphology seen in the light microscope (including size and shape), it was identified as *Pseudanabaena* cf. *moniliformis*. However, in the transmission electron microscope, thin sections revealed the thylakoids having a radial arrangement. It can therefore be distinguished from the *Pseudanabaena* group sensu Komárek and Caslavská (1991), which is characterized by a more or less concentric arrangement of the thylakoids. A detailed description of the ultrastructure including the cell wall and cellular inclusions is also provided. The phylogenetic relationship between this species and other cyanobacteria was investigated by sequence determination of the phycocyanin gene. Toxin production studies of strain HOs24 using ELISA and HPLC analyses showed it to produce six variants of microcystins.

PO.13-07
Mucilage phenomena in North Aegean Sea, Greece: another harmful effect of dinoflagellates?

Session: PO.13 - Regional events

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Mucilage phenomena are known in the Mediterranean Sea since the 18th century, while in Greece the first reports go back to the ’80s. Recently, the formation of mucilage has been associated with the dinoflagellates *Gonyaulax hyalina* and *G. fragilis* in Tasman Bay (New Zealand) and the Adriatic Sea (Italy), respectively. After successive mucilage formations and subsequent fisheries problems the last years in Greek coastal waters, samples were collected on a weekly basis from 14 sampling stations in Thermaikos Gulf (NW Aegean Sea) and sporadically from 20 sites in North Aegean Sea between June and October 2005 in order to investigate any causative organism for mucilage aggregations; *G*. cf.

fragilis was found from June to September coinciding with mucilage phenomena. Towards the end of summer, *Gonyaulax* cells were often found inside aggregations along with diatoms and heliozoans, while on many occasions only the thecae were found. Furthermore, the examination of preserved water samples collected in 2004 revealed that *G*. cf. *fragilis* was also present in summer 2004 when mucilage phenomena were also intense. The highest abundances of *Gonyaulax* ($7.92x10^3$ cells/L) were found on August 2005 in integrated water samples, while their abundance in 2004 did not exceed $1.40x10^3$ cells/L.

PO.13-32
DSP shellfish toxicity in relation to occurrence of *Dinophysis fortii* and *D. caudata* blooms

Session: PO.13 - Regional events

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Temporal and spatial distribution of *Dinophysis* species in relation to DSP shellfish toxicity was studied at six areas with 13 sampling stations along the eastern Adriatic coast from January 2001 to December 2005. Seven potentially toxic *Dinophysis* species were recorded including *D. acuta*, *D. acuminata*, *D. caudata*, *D. fortii*, *D. rotundata*, *D. sacculus*, and *D. tripos*. *Dinophysis* species differed in seasonal occurrence. *Dinophysis acuta*, *D. acuminata* and *D. sacculus* were more abundant in spring and autum. *Dinophysis rotundata* and *D. tripos* occurred in high abundance during winter and spring, while higher abundance of *D. rotundata* in NW Adriatic usually occurred in early summer. *Dinophysis caudata* and *D. fortii* showed maximum abundance in summer and autumn. *Dinophysis* species showed strong seasonal variability and different spatial distribution. Diarrhetic shellfish toxins outbreaks were usually associated with the presence of *D. fortii* and *D. caudata*. *Dinophysis fortii* was always associated with shellfish toxicity while *D. caudata* blooms were present during both periods with and without shellfish toxicity, indicating *D. fortii* as the most DSP toxic species in these areas.

PO.06-07
The emergence and dynamics of red tide blooms caused by *Cochlodinium polykrikoides* in the Peconic Estuary, NY, USA

Session: PO.06 - Population dynamics

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The dinoflagellate *Cochlodinium polykrikoides* is well known for forming harmful red tide blooms in Asian waters to the detriment of fisheries and aquaculture there. In the US, *C. polykrikoides* blooms have been less common. Here we report on the emergence of a *C. polykrikoides* blooms in the Peconic Estuary, NY, USA, during the late summer and early fall of 2004 and 2005. During both years, blooms achieved cell densities exceeding 10^7/L and chlorophyll levels exceeding 100µg/L throughout much of the estuary, although bloom waters were characterized by extreme patchiness. High-resolution spatial and temporal sampling during 2005 suggested

that the bloom was initiated in the upper tributaries of the Peconic Estuary in August, and progressively spread to the outer estuary in September. Aquacultured juvenile oysters (*Crassostrea virginica*) and wild juvenile and adult soft shell clams (*Mya arenaria*) experienced elevated mortality during the blooms, a finding particularly troubling in light of the ongoing multi-million dollar efforts to restore the Peconic Estuary's shellfish populations. Incubation experiments during bloom events suggested that both nitrogen and organic micronutrients (vitamins) may both play a key role in stimulating the growth of *C. polykrikoides* during bloom events.

PO.05-04

Nitric oxide synthase-mediated nitric oxide (NO) generation by harmful red tide phytoplankton, *Chattonella marina*

Session: PO.05 - Toxin analysis

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The harmful red tide phytoplankton species *Chattonella marina* is known for its potent fish-killing activity. Previous studies have demonstrated that *C. marina* produces reactive oxygen species (ROS), and a ROS-mediated ichthyotoxic mechanism has been postulated. In this study, we found that *C. marina* is producing relatively high levels of nitric oxide (NO) under normal growth conditions. We utilized a chemoluminescence (CL) reaction between NO and luminol-H_2O_2 to detect NO in a *C. marina* cell suspension. Significant CL was observed in a cell-number dependent manner, and it decreased after addition of carboxy-PTIO, a specific NO scavenger. The estimated level of NO produced by *C. marina* was higher than those of ROS. The NO generation by *C. marina* was also confirmed by a spectrophotometric assay based on the measurement of the diazo-reaction positive substances (NOx) and by fluorometric assay using a highly specific fluorescent indicator of NO. The NO level in *C. marina* was significantly reduced by L-NAME, a specific NO synthase (NOS) inhibitor, and the addition of L-arginine resulted in an increase in NO level, whereas $NaNO_2$ had no effect. These results suggest that NOS-like enzymes are mainly responsible for NO generation in *C. marina*.

PO.10-03

Growth and toxin production of the dinoflagellate, *Alexandrium minutum* (Dinophyceae) isolated from Tumpat Estuary, northeastern part of Peninsula Malaysia

Session: PO.10 - Ecophysiology & autecology

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The tropical estuarine dinoflagelallate, *A. minutum* Halim was used to determine the ecophysiological adaptation in relation to the temperate counterparts. This species has been frequently associated with incidence of paralytic shellfish poisoning (PSP) in Southeast Asia in recent years. The effects of irradiance and temperature on growth, nitrate assimilation and PST production were investigated in clonal batch cultures over the growth cycle. Growth rates increased with increasing temperature and irradiance. Growth was depressed at lower temperature (20 °C) and irradiance (40 µmol photons m^{-2} s^{-1}). The species showed no net growth at 10 µmol photons m^{-2} s^{-1} and a temperature of 15 °C, although cells remained alive. Cellular toxin quotas (Qt) varied in the range 10 – 42 fmol PST $cell^{-1}$. Toxin production rate, Rtox increased with elevated light at both 20 °C and 25 °C, with pronounced effect observed in the exponential phase (r^2 = 0.96). Rtox also increased significantly with increased temperature ($P < 0.05$). The ecotypic variations in growth adaptations and toxin production of this Malaysian strain may reveal a unique physiological adaptation of tropical *Alexandrium* species.

PO.14-15
Exterminating model of toxic microalgae by electrochemical method

Session: PO.14 - Mitigation

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Chemical methods to exterminate toxic microalgae have being developed; but application of the methods in river head areas and fish farms is restricted. Exterminating toxic cyanobacteria is important because of increasing concentrations of toxic compounds through the food chains. A method that does not use chemicals is preferred in riverhead areas and fish farms.
The electrochemical method is characterized by the elution of a metallic ion from the structures. Large amount of calcium and magnesium dissolved in water can be obtained easily from shells and used for the machine structure. Iron, aluminum, sodium and potassium are dissolved as ions in water, but their concentrations are low. Iron and aluminium chelates have effective cohesion effects but they spoil the taste of water.
The performance of the model device based on adsorption, precipitation (effect of cohesion) and surfacing was evaluated by using the solution in which several kinds of algae have been cultured. The toxic microalgae were adsorbed and concentrated by the device. Different methods to remove the chemicals and the elements discharged from toxic microalgae were tested.

PO.07-11
Impact of *Lingulodinium polyedrum* blooms on the northern coast of Baja California, Mexico

Session: PO.07 - Ecology and oceanography

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Red tides occur annually on the coast of Baja California (BC) and a phytoplankton monitoring program was therefore initiated in 2003 to detect potentially harmful algae before they impact fish farms. Phytoplankton samples were taken using a segmented pipe. The most important red tide event of the last 20 years occurred in spring/summer 2005 and the dominant species was *Lingulodinium polyedrum*. A maximum value of 17 500 000 cells/L was recorded for this species at Salsipuedes, BC, in August, while values of 4 500 000 and 4 990 000 cells/L were obtained at Puerto Escondido, BC, in June and July, respectively. In addition to the direct impact on the bottom fauna when *L. polyedrum* attained a density of 6 300 000 cells/L in a shallow area of Ensenada Bay in June, secondary effects were observed. In October, in Ensenada Harbour and Coral Marina, tons of anchovy (*Engraulis mordax*) were infected by *Acinetobacter vaumannii* and died. Subsequently, unusual tuna mortalities occurred in farms at different localities. Paralytic and amnesic shellfish poisoning were not detected in the dead tuna. The *L. polyedrum* bloom could have played a role in these complex toxic outbreaks.

PO.13-20
Harmful flagellates in the Nervion River Estuary

Session: PO.13 - Regional events

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Several potentially harmful flagellate species have been identified in the Nervion River Estuary, a eutrophic warm temperate estuary which has experienced in the last years a marked improval of water quality. Species belong mostly to Dinophyta, including the genera *Akashiwo*, *Alexandrium*, *Dinophysis*, *Karenia*, *Karlodinium*, *Lingulodinium*, *Pfiesteria*, *Prorocentrum* and *Protoceratium*, and to a lesser extent to Heterokontophyta, represented by *Chattonella*, *Fibrocapsa* and *Heterosigma*, or Haptophyta, with harmful representatives of *Chrysochromulina*, *Phaeocystis*, *Platychrysis*, *Pleurochrysis* and *Prymnesium*. Some species such as *Heterosigma akashiwo* and *Phaeocystis globosa* appear recurrently and, in many cases, at elevated densities. The remaining species appeared in very low concentrations or were identified in cultures of natural samples together with the dinoflagellates *Karlodinium micrum* and *Karlodinium* sp., and the haptophytes *Prymnesium parvum*, *Chrysochromulina brevifilum*, *Pleurochrysis pseudoroscoffensis* and *Platychrysis pienaari*. In this study, we present the relationship between the physical environment, with emphasis on haline or thermal stratification, and the presence of these harmful species along the longitudinal axis of the estuary.

PO.13-61
Bloom-forming *Pseudo-nitzschia* species (Bacillariophyceae) from the southeastern coast of Russia: morphology, distribution and toxicity

Session: PO.13 - Regional events

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A survey for species of the potentially toxic diatom genus *Pseudo-nitzschia* was carried out on the south-eastern coast of Russia: Peter the Great Bay (within the Sea

of Japan) and Aniva Bay (within the Sea of Okhotsk). The bloom-forming *Pseudo-nitzschia* species found were *P. multiseries*, *P. multistriata* and *P. calliantha*, as identified by TEM and SEM. Morphometrics, taxonomic discussion and distribution patterns are presented for these three species. We document, for the first time, the toxicity of a *Pseudo-nitzschia* species isolated from Russian waters. The highest domoic acid concentration was 3,780 ng ml^{-1} (11 pg $cell^{-1}$), in a 23 day-old culture of *P. multiseries* from Amursky Bay. No domoic acid was detected (<2 ng ml^{-1}) in the cultures of *P. calliantha* and *P. multistriata* from the same location. No poisoning events or mass mortality of sea birds or mammals have yet been reported. However, regular phytoplankton monitoring is needed to detect the appearance and density of potentially toxic *Pseudo-nitzschia* species along the southeastern coast of Russia.

PO.05-25

Characteristics of PSP-toxin profiles in bivalves from Japanese coastal waters

Session: PO.05 - Toxin analysis

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Contamination of shellfish by PSP toxins occurs all over Japan from Hokkaido to Okinawa. So far, 4 species of dinoflagellates have been identified as causative organisms. Since the basic information on the toxins is important for the application of new analytical methods to the monitoring program, toxic samples were systematically collected from the major shellfish growing areas and analyzed by HPLC. More than 300 samples showing toxicity close to the regulation limit were selected for the analysis.
Shellfish contaminated by *Alexandrium tamarense* in northern Japan showed similar toxin profiles, with GTX1-GTX4 and neoSTX as major toxins. However, those related to autumn blooms of *A. catenella* showed a large proportion of C1/C2 toxins. Mussels, clams and oysters from western Japan showed much more variation in toxin profiles. Especially, those contaminated by *Gymnodinium catenatum* were characterised by a large proportion of N-sulfocarbamoyl toxins such as C1/C2, GTX5 and GTX6. Despite great variation in toxin profiles, estimated toxicities from HPLC data agreed well with the results of the mouse bioassay. However, in low toxicity samples HPLC-based toxicities always exceeded those of the bioassay, due to the matrix effect in the bioassay.

PO.14-07

Looking into the use of clay to control *Pyrodinium* blooms in the Philippines

Session: PO.14 - Mitigation

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Pyrodinium blooms in the Philippines have resulted in more than 2100 cases of paralytic shellfish poisoning due mainly to ingestion of the vector species, *Perna viridis*. Some efforts have been made to respond to this toxic outbreak. This study is the first attempt to examine the use of clay to control a *Pyrodinium bahamense* bloom. The removal efficiency of

local clays (ball clay, brown bentonite and white clay) and marine sediments was determined in a microscale set up. Ball clay exhibited high removal efficiency of >99% at final concentration of 1 g/L. Removal efficiency was 69% for brown bentonite, 48% for white clay, and 59% for marine sediments. Cell removal of ball clay was consistent from 2.5 to 24 h after clay addition. The effectiveness of ball clay was also tested against other HAB species, *Chatonella marina* and *Amphidinium carterae,* showing cell removal of 25% and 50%, respectively. Mesoscale experiments will be done for the efficacy of ball clay on larger volume and under turbulent condition. The effect of ball clay addition on seawater chemistry showed no change in ambient ammonia concentration but nitrate decreased after 5 and 24 h of clay addition. Results for nitrite and phosphate varied between two runs.

PO.13-67
Harmful algal blooms along the Kerala coast, southern India

Session: PO.13 - Regional events

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During 9 – 21 September 2005, the coastal regions from Kollam to Vizhinjam of Kerala experienced massive fish kills followed by obnoxious and nauseating smell for about a 100-km stretch along the coast. The coastal waters had strong discolouration and high concentration of *Cochlodinium polykrikoides*. There was a drastic decline in the concentration of oxygen, nitrite, nitrate and phosphate during the initial bloom period. The concentration of the dinoflagellates ranged from 2.5 to 4.7 X 10^6 cells L^{-1}. This resulted in a serious health hazard as more than 200 persons, especially school children were admitted in hospitals. It also adversely affected the mussel and fin- fish fishery along the coast. The mouse bioassay revealed no toxicity in mussels. Subsequent to this event, another massive fish kill had occurred at Varkala on 27 September 2004, which coincided with high concentration of *Ceratium furca* (1.6 X 10^4 cells L^{-1}) and *Cochlodinium polykrikoides* (2.2 X 10^4 cells L^{-1}). The HAB is a recurring phenomenon along the Kerala coast. Currently a national coordinated monitoring programme 'Harmful Algal Blooms in the Indian EEZ' is being carried out along the southern coasts of India.

PO.14-03
Modified local soils/sediments for HAB removal and macrophytes restoring in shallow lakes

Session: PO.14 - Mitigation

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The major concerns for mitigating HAB using clays in shallow lakes are low HAB removal efficiency for most clays in fresh waters; high clay loading and operating costs; ecological risks for adding exotic clays; re-suspension of algal cells and increased anaerobic release of nutrients/pollutants from the sediment; and the inherent incapability for preventing the occurrence of HAB from long point of view. We proposed a MLSIER (PCT patent filed) technology (modified-local-soils/sediments induced ecological restoration), which made it possible to solve all these problems. In the study of the MLS technique, it was found that some natural netting and bridging materials, such as chitosan, turned many solids, such as local soils/sediments, into highly effective flocculants in removing cyanobacterial blooms fromn fresh waters (1-3). When MLS was further modified with macrophyte seeds (MLSIER), the short-term effect of algal removal triggered a long-term macrophyte growth (the latter would otherwise die in HAB waters), which permanently fixed the cells and nutrients into the sediments.

1. Pan G, Zhang MM *et al*., Environmental Pollution, 2006, 141 (2): 195.
2. Zou H, Pan G *et al*., Environmental Pollution, 2006, 141 (2): 201.
3. Pan G, Zou H *et al*., Environmental Pollution, 2006, 141 (2): 206.

PO.10-18
Trying to cultivate *Dinophysis acuminata*, a dinoflagellate causing diarrhetic shellfish poisoning

Session: PO.10 - Ecophysiology & autecology

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The dinoflagellate genus *Dinophysis* includes several toxic species which cause diarrhetic shellfish poisoning, and no species of the genus has yet been established in culture. While the plastids in *Dinophysis* are known to be of cryptophyte or haptophyte origin, whether they are kleptoplasts and how they are acquired by *Dinophysis* still remain unresolved. To address these issues, we tried to establish *D. acuminata* in laboratory culture. The results will be discussed in context of biology and plastid evolution of *Dinophysis* species.

PO.12-08
Morphological characteristics and life cycle of the diatom *Thalassiosira* cf *stellaris*

Session: PO.12 - Taxonomy and phylogeny

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Strains of *Thalassiosira stellaris* and *Thalassiosira* cf *stellaris* were isolated off the coast of Wando, Korea. *T.* cf *stellaris* resembles *T. stellaris* in many morphological characteristics such as cell length, fascicular distribution of areolae, and the number and interval of strutted processes on the margin and middle of the valve face. *T.* cf *stellaris* differs in lacking dot-shaped small processes on the valve face, and the strutted processes are surrounded by four additional small areolae. *T*. cf *stellaris* displayed typical oogamous reproduction. In a phase of vegetative multiplication, several cells were connected by mucous threads to build up short chains in which each cell stands apart at regular intervals, but during and/or just before sexual reproduction, the cells were closely connected to neighbouring cells producing chains without mucilaginous threads. Cell counts of single cells decreased with growth of the filamentous chains. These results imply that the filamentous chains might play the role of oocyte and the single cells may be spermatocytes. In spite of identical culture conditions of *T*. cf *stellaris* and *T. stellaris*, sexual reproduction was observed only in *T*. cf *stellaris*.

PO.10-41
Examination of the cell cycle, growth rate, and meiosis of *Karlodinium* spp. by flow cytometry

Session: PO.10 - Ecophysiology & Autecology

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The DNA content, DNA synthesis cycles, and growth rates of different strains of *Karlodinium veneficum* and *K. armiger* were studied in culture by flow cytometry. A 3-fold difference in DNA content was found between *K. veneficum* and *K. armiger*, indicating a significant difference in genome size. *K. veneficum* strains had a diel periodicity of DNA synthesis (S) and cell division (G2M) that was phased with the photocycle. Cells with 1C DNA (G1) began DNA synthesis (S) during the latter half of the light period, then entered G2M and divided during the dark period. Variation among *K. veneficum* strains was found in the duration of the S phase (3.2-8.4 h) and G2M phase (2.0-12.7 h). Potential growth rates derived from cell cycle data ranged from 0.55 to 1.03 divisions day^{-1}, and were higher than net growth rates calculated from cell numbers. In most strains, a distinct subpopulation of cells with a single nucleus containing 4C DNA also occurred during the dark period. Based on observations of the sexual life cycle of *K. veneficum*, it is proposed that these 4C DNA cells were zygotes undergoing meiosis. This potentially co-occurring meiotic cycle could impact determinations of the mitotic cell cycle of *Karlodinium*.

PO.08-20
Impacts of the toxic dinoflagellate *Alexandrium monilatum* on three ecologically important shellfish species

Session: PO.08 - Toxicology

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Alexandrium monilatum is a toxic dinoflagellate that forms blooms mostly in the Gulf of Mexico. Toxic *A. monilatum* has been linked to fish and invertebrate kills, and produces endotoxins with hemolytic and neurotoxic properties. We experimentally assessed responses of ecologically important shellfish to toxic *A. monilatum* (AMO3 isolate). Grazing studies were conducted with eastern oysters (*Crassostrea virginica*), northern quahogs (*Mercenaria mercenaria*) and green mussels (*Perna viridis*), all of which inhabit areas where *A. monilatum* blooms occur. Clearance rates of each shellfish species were depressed when exposed to toxic *A. monilatum* alone or with nontoxic Instant Algae® *Pavlova*, in comparison to control animals that were fed benign algal prey. Exposure to *A. monilatum* also significantly decreased shellfish

valve gape. Bioassays were conducted to test survival of larval shellfish exposed to *A. monilatum* as whole, unconstrained cells, cells held in dialysis tubing, or sonicated cells. Sonicated *A. monilatum* caused a significant decrease in larval survival, in comparison to survival of the control larvae that were tested with nontoxic *Alexandrium tamarense*. Overall, these data indicate that *A. monilatum* blooms can adversely affect shellfish survival by reducing clearance rate and valve gape, affecting food intake, and inducing larval mortality.

PO.13-53
Early detection and intensive monitoring during an unusual toxic bloom of *Gymnodinium catenatum* advected into the Galician Rías (NW, Spain)

Session: PO.13 - Regional events

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Cooperation between Galician and Portuguese monitoring programs allowed an early alert of a *Gymnodinium catenatum* bloom in the Galician Rías a month before the PSP toxin accumulation in mussels. Both programs detected isolated chains of *G. catenatum* from July 2005. The Portuguese monitoring system warned about high concentrations of *G. catenatum* on the Lisbon shelf in September and the progressive increase in the concentrations northward thereafter. From the near real-time sea surface temperature fields, it was inferred that a northward coastal current was present during this period.
On October 24th, a strong downwelling (Iw < -1000 $m^3 \cdot s^{-1} \cdot km^{-1}$) event occurred and surface waters warmer (>16.5 ºC) and less saline (35.2) flowed into the Rías, advecting a *G. catenatum* population (max. 1.7 x10^5 cells·L^{-1}). Data from a lagrangian drifter (NOAA/CoastWatch) confirmed the presence of a northward coastal current (Vel ~0.2-0.6 $m \cdot s^{-1}$) on these dates. Temporal fluctuations in *G. catenatum* bloom dynamic followed the upwelling-downwelling events. High cell concentrations persisted until December, decreasing gradually until spring 2006. After ten years of no *G. catenatum* blooming in Galician Rías, this intense and persistent episode caused bans on mussel culture areas (average production>2x10^5 tons·$year^{-1}$) until mid-February, having an important social and economic impact.

PO.10-45
Carbon dioxide production during an exceptional dinoflagellate bloom at Todos Santos Bay, Baja California, México

Session: PO.10 - Ecophysiology & autecology

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During an exceptional dinoflagellate bloom in 2005 at Todos Santos Bay, Baja California (perhaps the most intense bloom during the last 45 years), a 2-day survey cruise was carried out to study the impact of this event on the CO_2 ocean-atmosphere flux. Continuous measurements of carbon dioxide

partial pressure (pC O_2sea) along the sea surface were made, pCO_2sea average values of 600 ?atm were measured, inducing an ocean-atmosphere flux of 13.55 mmol CO_2 m^{-2} d^{-1}, our estimations revealed that the Todos Santos Bay exported at least 97 ton CO_2 per week to the atmosphere during the bloom. When the bloom began, the phytoplankton community was dominated by thecate dinoflagellates (*Lingulodinium polyedrum*, *Ceratium furca*, *C. fusus*, *Prorocentrum micans* and *Dinophysis* spp.) with maximum cell counts of 7 x 10^6 cells L^{-1}. At the end, the dominant species were *L. polyedrum* together with non-thecate dinoflagellates (*Gymnodinium* spp., *Gyrodinium* spp. and *Amphidinium* spp.) and the diatom *Cylindrotheca closterium*. Inorganic nutrients, oxygen, temperature and chlorophyll 'a' fluorescence profiles were also measured. A conceptual model is proposed in which the autotrophic respiration and bacterial oxidation of organic matter controls the pCO_2sea levels.

PO.15-05
Ribosomal DNA quantification in the dinoflagellates *Alexandrium catenella* and *Alexandrium taylori* for application in real-time PCR-based monitoring

Session: PO.15 - Monitoring

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Several molecular methods for monitoring of HAB species have been recently developed. In particular, quantitative real-time PCR can be used for the detection and quantification of genetically distinct HAB species in environmental samples using a standard curve as reference. The standard usually consists in the target sequences of rDNA genes cloned into a plasmid, or in DNA extracted from a known amount of cultured target cells. When a plasmid is used as standard, it is essential to know the amount of the target rDNA copy number per cell. In this study, the rDNA content of the HAB species *A. catenella* and *A. taylori* was estimated by real-time PCR using specific primers designed on the 5.8S rDNA region, and standard curves constructed with serial dilutions of the cloned target sequences. The data were useful for quantification of *A. catenella* and *A. taylori* in bloom samples using the real-time PCR-based approach. While *A. taylori* cell quantification was generally in agreement with microscope cell count, *A. catenella* cell quantification was underestimated compared to standard counting method. This could be partially explained by the presence of a pseudogene in the '*A. tamarense*/*catenella*/*fundyense*' complex. Further experiments will be needed in order to address this issue.

PO.06-15
An investigation of the relationship between *Pseudo-nitzschia* species and domoic acid in *Mytilus* sp. in the Fal Estuary, UK

Session: PO.06 - Population dynamics

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An 18-month detailed time series has been constructed for the occurrence of *Pseudo-nitzschia* species and domoic acid in *Mytilus* sp. from the Fal Estuary, UK. *Pseudo-nitzschia* species were regularly observed with four *Pseudo-nitzschia* species isolated into culture; *P. fraudulenta*, *P*. cf. *pseudodelicatissima*, *P. multiseries* and *P. pungens*, of which *P. multiseries* was confirmed to produce domoic acid. However, domoic acid was only detected in shellfish during a peak in *Pseudo-nitzschia*, when TEM analysis confirmed the dominant species to be *P*. cf. *pseudodelicatissima*. Spatial and temporal data indicated that these cells entered the estuary from the seaward source (i.e. the English Channel). There were also periods where *Pseudo-nitzschia* species occurred without the presence of domoic acid in shellfish. The presence of a range of non domoic acid producing *Pseudo-nitzschia* strains/species in the Fal Estuary could explain non correlation between cell abundance and domoic acid concentration in shellfish in this region. In addition, the use of size classing of *Pseudo-nitzschia* cells provided limited capability in aiding species prediction and therefore has restricted capability in the prediction of domoic acid accumulation in shellfish in this region.

PO.08-06
Cyanobacterial toxins as triggers for oxidative stress in plants

Session: PO.08 - Toxicology

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Cyanobacterial toxins have been shown to have adverse effects on plants, both terrestrial and aquatic. Microcystins are cyclic heptapeptides and the main group of cyanotoxins. During exposure of plants to cyanobacterial toxins the formation of reactive oxygen species can occur. These reactive oxygen species have strong reactivity and are able to interact with other cellular compounds like lipids, proteins and DNA.
Plants do have an effective antioxidative system which will limit the negative effects caused by reactive oxygen species. Seedlings of *Alfalfa* exposed to purified cyanobacterial toxins and cell-free cyanobacterial crude extract showed uptake of toxin, and adverse effects on germination, growth and root development. Antioxidative enzymes, such as superoxide dismutase (SOD) and catalase were examined at the protein- as well as on the gene level.

PO.13-63
First evidence of spirolide accumulation in northwestern Adriatic shellfish

Session: PO.13 - Regional events

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In autumn 2003, shellfish samples from the Northwestern Adriatic Sea, collected during the routine monitoring programme, tested positive by mouse bioassay for lipophilic toxins (Yasumoto, 2001). Unusual symptoms and the extremely short survival times (as low as 5 min), alerted the monitoring operators, suggesting the presence of compounds previously undetected in Italy. During the event, the dinoflagellate *Alexandrium ostenfeldii* reached its maximum concentration of 15,612 cells/L. The microalga, isolated and grown in culture, was demonstrated to produce mainly spirolide 13-desmethyl C but no PSP toxins (Ciminiello, 2006). Here we report on the first evidence of spirolide accumulation in Adriatic mussels. Analyses were carried out by liquid chromatography tandem mass spectrometry.

PO.05-34
Use of electrospray tandem mass spectrometry for identification of microcystins during a cyanobacterial bloom event

Session: PO.05 - Toxin analysis

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Drastic environmental conditions such as elevated temperature, abrupt pH variation, low turbulence, and high nutrient inputs can enhance the development of toxic cyanobacterial blooms in lakes and reservoirs. This study describes the occurrence of four microcystin variants (MC) in a bloom in the eutrophic reservoir Billings, in Sao Paulo City. The bloom sample was collected in October 2003, and *Microcystis* was the main genus found. The MC were separated and purified by reverse phase high performance liquid chromatography (RP-HPLC). Their structures were elucidated by electrospray ionization tandem mass spectrometry (ESI-MS/MS) and four variants were determined: MC-RR, MC-LR, MC-YR, and MC-hRhR. MC-hRhR is described for the first time as a new variant of MC with two homoarginines at positions 2 and 4 in its structure. ESI-MS/MS analysis thus provides a powerful and convenient tool for the determination of variants of MC. These results represent an important contribution to the knowledge of the biochemistry of toxic cyanobacteria and their toxins, specifically in Sao Paulo State.

PO.04-03
Effects of harmful algae on rotifer feeding behaviour and reproduction: *Karenia brevis* uses chemical defense to deter grazers

Session: PO.04 – Food chains

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Bloom-forming phytoplankton may avoid becoming prey for zooplankton because phytoplankton cells are toxic, unpalatable, or nutritionally inadequate to support zooplankton growth or reproduction. Using laboratory feeding assays, we

tested the effects of three dinoflagellates, *Karenia brevis*, *Prorocentrum minimum*, and *Peridinium* sp., on the feeding behaviour and fitness of the estuarine rotifer *Brachionus plicatilis*. *Prorocentrum minimum* and *Peridinium* sp. were consumed at similar rates as a control food (*Rhodomonas lens*), but the consumption of *P. minimum* or *Peridinium* sp. resulted in reduced rotifer reproduction. In contrast, *K. brevis* cells and extracts deterred rotifer feeding, indicating that *K. brevis* is chemically defended against rotifer grazing.

PO.13-60

Killing effect of heterotrophic bacteria on bloom-forming phytoplankton species from the coastal area of Thailand

Session: PO.13 - Regional events

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The relationship between the abundance of heterotrophic bacteria and the bloom-forming species of diatoms *Skeletonema costatum* and *Chaetoceros* spp., cyanobacteria *Oscillatoria* spp. and dinoflagellates *Noctiluca scintillans* and *Ceratium furca* was investigated from natural samples collected from the coastal area of Bangpra, in the eastern part of the Upper Gulf of Thailand from January 2003 to January 2004. The temporal variation in the abundance of heterotrophic bacteria showed the same trend as those of the dominating phytoplankton species, *S. costatum*, *Chaetoceros* spp. and *O. erythraeum*. The effects of natural bacterial populations on representative phytoplankton speies such as *O. erythraeum*, *C. curvisetus* and S. costatum studied from May to July 2003 (early rainy season) were not clearly established. However, three clonal cultures of bacteria, *Bacillus* sp., *Pseudomonas* sp. and unidentified pale yellow bacterial colony, isolated from seawater in June 2004 at the concentrations higher than 10^5 cells/ml, inhibited growth of *C. curvisetus* and *S. costatum*. The growth of a natural population of the dinoflagellate *Noctiluca scintillans* was also affected by *Bacillus* sp. concentrations higher than 10^5 cells/ml and the pale yellow bacteria and *Pseudomonas* sp. of the concentrations higher than 10^4 cells/ml.

PO.05-11

Variability of particulate and dissolved lipophilic toxins during and after *Dinophysis acuta* growth in the Galician Rias

Session: PO.05 - Toxin analysis

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In autumn 2005, moderate numbers of *Dinophysis acuta* in the Galician Rías Baixas were found from early September, and record concentrations were reached the second week of November. Toxin profiles and toxin content of lipophylic toxins (okadaiates, pectenotoxins) in plankton net-hauls (40-77 µm size fraction, pumped from 3-5 m), picked *Dinophysis acuta* cells, and seawater (adsorbed with DIAION HP20 resins) were monitored weekly at a fixed station in Ria de Pontevedra. A more

intense monitoring (every 2h for 24 h) of toxins in *Dinophysis* was carried out during a cell cycle study in November. *Dinophysis* cells and net-hauls contained OA, DTX2, OA diol-esters and PTX2; the same toxins plus PTX2SA and PTX1, but no diol-esters were dissolved in seawater and adsorbed by the resins. Toxins dissolved in seawater were detected until the end of January 2006, at least one month after the disappearance of *Dinophysis* cells. Seasonal and cell cycle variability in toxin content per cell (up to 95 pg of okadaiates and 38 pg of pectenotoxins) will be discussed in relation to cell size, phase of the population, and stages of the cell cycle. The origin and fate of toxins released in the seawater are discussed.

PO.08-07
Preliminary cultures *in vitro* of potentially toxic epiphytic dinoflagellates from a northern Philippine reef

Session: PO.08 - Toxicology

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Studies were conducted on laboratory cultures of the epiphytic dinoflagellates *Gambierdiscus* sp., *Ostreopsis* spp., and *Prorocentrum* spp. collected from depths of 1-1.5m at Lingsat Reef, La Union, Philippines. The initial culture utilized the ES1 medium in stoppered 15-ml culture tubes. At a temperature of 27±20 °C, irradiance 3,200 lux, salinity 23 ppt and pH 8.0, *Gambierdiscus* sp., starting from 8 cells/ml, reached a density of 15 cells/ml in 23 days, *Ostreopsis* spp. from 9 cells/ml to 22 cells/ml in 16 days, *Prorocentrum* spp. from 7 cells/ml to 190 cells/ml in 51 days. The cultures maintained themselves as follows: *Gambierdiscus* up to the 48th day, *Ostreopsis* up to the 57th day and *Prorocentrum* up to more than 150 days. This study was financed by the U.P. Natural Sciences Research Institute.

PO.02-01
Comparative genomic analysis of DNA fragments from a toxic cyanobacterial bloom

Session: PO.02 - Genomics

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The variety of secondary metabolites found in cyanobacterial blooms and the impacts they have on human health warrants large-scale approaches into understanding the ‘bigger picture’ of cyanobacterial bloom population structure and function. A metagenomic approach, which enables studies on genomes of mixed natural communities, has been utilized to overcome this problem of microbe 'unculturability'. We have prepared a bacterial artificial chromosome library (BAC) from the DNA extracted from a naturally occurring toxin-producing cyanobacterial bloom. The 3000 clone BAC library, which is the first report of a BAC library constructed from cyanobacterial bloom DNA, had an average insert size of 30kb with a number of clones containing inserts >50kb. From metagenomic library 16S rDNA PCR screens and a random BAC-end sequencing survey, 10 clones were selected for insert sequencing completion and analysis. Approximately 200 kb of cyanobacterial bloom metagenome has currently been sequenced and gene-finding and annotation analysis has identified and examined over 150 open reading frames (ORF's). This information has provided an insight into the physiology of a toxin-producing cyanobacterial bloom metagenome and identified proteins of interest both ecologically and in terms of potential in industrial processes.

PO.11-03
Species-specific allelopathic interactions involving the red tide dinoflagellate *Karenia brevis*

Session: PO.11 - Allelopathy

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Competition is one of the dominant processes structuring marine pelagic communities. Allelopathy, a mechanism of interference competition by which one species inhibits another via excretion of natural products, may allow a phytoplankton species with poor nutrient acquisition abilities to be a dominant competitor, and may also contribute to the formation of harmful algal blooms (HABs) by excluding non-bloom-forming species. We have found that allelopathy is common among Gulf of Mexico phytoplankton species, but that compounds other than well-known HAB neurotoxins are typically involved. Bioassay-guided fractionation, followed by spectroscopic identification of allelopathic compounds, is being used to identify the molecular structures of allelopathic compounds produced by the red tide dinoflagellate *Karenia brevis*. Allelopathy may also be inducible, whereby the presence of a specific competitor led to increased allelopathy by K. brevis.

PO.11-04
Phaeocystis globosa Scherffel, its haeomolytic and allelopathic effects

Session: PO.11 - Allelopathy

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Phaeocystis globosa blooms were recorded in China since 1997. The physiological characteristics of this species, as well as characters of its haemolysins were described. Haemolytic substances produced under nutrient limitation showed an allelopathic response to adverse conditions. Potential allelopathic effects on three harmful bloom algae, *Prorocentrum donghaiense*, *Chattonella marina* and *Chattonella ovata* were studied. Growth of *C. marina* and *C. ovata* was inhibited considerably when co-cultured with *P. globosa* or cultured in cell-free spent medium. Haemolytic extracts from *P. globosa* cells in the senescence phase had a similar inhibitory effect on the three HAB species. The brine shrimp *Artemia salina* was less affected by *P. globosa*. The results indicate that *P. globosa* has an allelopathic effect on microalgae, and this may explain its superior competitive abilities. Since addition of the haemolytic toxins from *P. globosa* gave similar effects on the algae compared to spent media, these compounds could be involved in the allelopatic action.

PO.13-14
Temporal and spatial distribution of *Pseudo-nitzschia* species (Bacillariophyceae) along the NE coast in Catalan coastal waters, NE Spain (Mediterranean Sea)

Session: PO.13 - Regional events

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Temporal and spatial distributions of potentially toxic species of the genus *Pseudo-nitzschia* were studied in 2005 along the Catalan coast (NW Mediterranean). Electron microscopic techniques revealed the presence of *Pseudo-nitzschia brasiliana*, *P. calliantha*, *P. delicatissima*, *P. fraudulenta*, *P. multistriata* and *P. pungens*. The two species *P. pungens* and *P. fraudulenta* were mainly found in the northern region, with maximum concentrations between February and May ($3.2 \cdot 10^4$ and $5.04 \cdot 10^5$ cells L^{-1}, respectively). *Pseudo-nitzschia delicatissima* and *P. calliantha* were found at the northern stations between February and March ($6.08 \cdot 10^5$ to $4.56\ 10^5$ cells L^{-1}, respectively), and appeared at the southern locations between August and November ($7.92 \cdot 10^5$ to $3.37 \cdot 10^5$ cells L^{-1}, respectively). *Pseudo-nitzschia brasiliana* and *P. multistriata* were only occasionally detected in the southern region, with maximum concentrations between August and September ($2.5 \cdot 10^5$ and $4.16 \cdot 10^6$ cells L^{-1}, respectively). The sum of the identified *Pseudo-nitzschia* species showed high values (up to $4.16 \cdot 10^6$ cells L^{-1}) during most of the year, the population initiated a sharp decline at all stations when water temperature increased beyond 21°C (April to May). Cultured isolates were submitted for HPLC analysis of pigments to evaluate the possibility of monitoring natural populations by a chemotaxonomic approach.

PO.05-41
Emerging algal toxins in Canada

Session: PO.05 - Toxin analysis

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Prior to 1987, the only phycotoxins of concern to Canada were those responsible for PSP. In 1987, domoic acid caused a serious incident of ASP in Eastern Canada and in 1992, the toxin DTX1 was implicated in a DSP event in Nova Scotia. In 1995, an entirely new class of toxins, spirolides (SPX), were identified in shellfish that caused anomalous mouse deaths in the lipophilic toxin assay. Following these incidents, a more proactive approach was taken, based on comprehensive surveillance of shellfish and plankton for other toxin classes using LC-MS. This led to the detection of PTX2 in net tow samples and PTX2sa in mussel tissue in Nova Scotia in 2000. More recent discoveries include gymnodimine, DTX1 and PTX2 in British Columbian samples in 2003; PTX2, DTX and SPX throughout Eastern Canada; and yessotoxin (YTX) in mussels from Newfoundland in 2004. Additionally, with the implementation of solid phase toxin tracking (SPATT), YTX, PTX11 and azaspiracids (AZAs) have been detected and confirmed in Nova Scotia. Causative organisms have been identified for most of these toxins. This poster will review these discoveries and demonstrate the capacity of LC-MS and SPATT techniques guide decision making for traditional monitoring programs.

PO.13-91

Toliara reef-lagoon ecosystem quality and state of health

Session: PO.13 – Regional events

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The Toliara region of Madagascar is concerned with ciguatera seafood poisoning. The Toliara region is a tourist development area with the Great Reef of Toliara as one of the main attractions. The objectives of this study is (i) a diagnosis of the Toliara reef-lagoon ecosystem quality and state of health, (ii) an identification of sources of pollution and other threats, (iii) delivery of advice for measures to eliminate or reduce pollution. A suite of microbiologic parameters are monitored in time and space, including enteric bacteria, phytoplankton, and microphytobenthos. The bacterial charge to this ecosystem is high particularly along the seaside, in the sediments, and in front of settlements. Open waters seems less charged in bacteria. Phytoplankton shows presence of indicators of environmental degradation such as toxic dinoflagellates and cyanobacteria with an abundance peak during the hot season, corresponding to the appearance of seafood poisoning incidents. Benthic dinoflagellates are abundant during the hot period of the year between January and March. They are present on different types of substrate, but macroalgae and especially dead coral are preferred.

PO.13-37

First record of *Gymnodinium catenatum, Gambierdiscus toxicus and Pyrodinium bahamense* var. *compressum* in the northern part of Luanda Coast (Angola)

Session: PO.13 – Regional events

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Phytoplankton samples were collected each month from July 2003 to October 2004 in four stations along the northern area of Luanda coast, with the aim of characterizing the dynamics of phytoplankton community. During this period two peaks of phytoplankton abundance were found, between August and October in both years. The results show that higher phytoplankton abundance in northern Luanda cost area occurs during the transition of the winter (dry season) to summer (warm season). The appearance of harmful algal blooms occurred concurrently with the phytoplankton density peaks in August. The HABs found in 2003 were dominated by dinoflagellates *Pyrodinium bahamense* var *compressum*, and

Gymnodinium catenatum. In 2004 were also registered the occurrence of *Gambierdiscus toxicus.* During this last years, in August, were observed the highest peak of densities of these species namely 265463 cells/L of *Pirodinium bahamense* var *compressum*, 164305 cells/L of *Gambierdiscus toxicus* and 158333 cell/L of *Gymnodinium catenatum.* The occurrence of these species may be related to the environmental parameters such as temperature and salinity.

PO.10-13
Biology and seasonal distribution of *Hermesinum adriaticum* in the New River of North Carolina

Session: PO.10 - Ecophysiology & autecology

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The New River in southeastern North Carolina is a brackish water system having a variety of microalgae that annually form blooms. Some bloom species are considered toxic; however one rare microorganism that may play a significant role in the river's ecology has been overlooked. An ebridian, *Hermesinum adriaticum* is found frequently in the New River during months when water temperatures are above ~22 °C. This unicellular organism contains an internal skeleton protruding from both ends of the cell making the cell about 50μm by 20μm. Cell populations in the New River commonly reach 40,000 cells/L and once a population of 150,000 cells/L was observed at French Creek. Lugols preserved monthly water samples from seven different stations in the New River collected from 2001-2006 were observed to determine annual abundance and seasonal distribution of *H. adriaticum*. From live samples and cells cultivated in the laboratory, observations were made on live material to determine growth characteristics, including life cycle stages, feeding and growth behavior. Also, detailed studies were conducted using both scanning and transmission electron microscopy. SEM was used to examine external morphological changes including possible loss of skeletons, while TEM was used to study nutrition, possible endosymbiotic relationships, and internal structural changes.

PO.13-38
Diarrhetic shellfish toxins at the Swedish West Coast 1987-2005

Session: PO.13 - Regional events

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Diarrheic shellfish toxins have occurred and been regularly chemical monitored in blue mussels (*Mytilus edulis*) at the Swedish west coast from year 1987. In general there is a seasonal variation of DSTs in mussels with concentrations low from March to August and high from October to December. Peaks above the limit for consumption have in some years also occurred in late June to late July. A rapid intoxication vs. slow detoxification of mussels is common. Temporal and regional differences are large. Mussels grown in areas exposed to the open sea have in general higher toxin values than semi-enclosed fjord systems. There is also a considerable variation in toxin levels between years. In 1997 mussel farmers experienced very low levels. In autumn 1989 to spring 1990 and in early autumn 2000 to early 2001, high levels were recorded during 26 weeks in a row. In neither of these cases toxicity could be linked to unusually high or low levels of *Dinophysis*. The Kolje Fjord region had low levels of toxins until year 1998, despite regular recordings *Dinophysis* in the area. Today mussels grown and harvested in this area have similar toxin levels as mussels from other fjords in the region.

PO.13-36

An investigation into the ecotoxicology of different strains of *Lingulodinium polyedrum* from the Portuguese coast

Session: PO.13 - Regional events

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Lingulodinium polyedrum is known to be a common species along the Portuguese coast. In this region the first record that associates *L. polyedrum* with red-tide events dates back to 1944. This species is frequently associated with shellfish contamination and fish mortality, and yessotoxin production has already been detected in *L. polyedrum* from other areas. Blooms occurred along the south and west coast in the summers of 2004 and 2005, leading to extensive precautionary beach closures. However, prior to the present work, no information was available regarding the toxicity of Portuguese *L. polyedrum* populations. Here, we investigate the potential yessotoxin production by different strains isolated from the west coast and south coast. Toxin production was studied in the lag, exponential, stationary and death phase, using HPLC-MS as a detection method. Preliminary results suggest that none of these strains are yessotoxin producers and the strains are not toxic in any of the examined growth phases. Intraspecific differences in growth rates were also investigated and preliminary results will be presented.

PO.06-08

Time-series study of the occurrence of dinoflagellate cysts in surface sediments from a warm temperate region (Cascais Bay, Portugal)

Session: PO.06 - Population dynamics

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A five-year survey was conducted between January 2000 and November 2005 to assess the seasonal occurrence of dinoflagellate cysts in surface sediment samples from Cascais Bay, Portugal. A total of 53 cyst morphotypes were found. Results indicate that the overall cyst production follows a seasonal pattern, with maximum abundance of cysts with cell contents occurring in late autumn. However, individual cyst morphotypes and dominance of taxonomic groups present a variable year-to-year behaviour, associated with major shifts on the species composition of the community, and some species showing cyst 'blooms' at time intervals larger than 5 years. Cysts of the toxic species *Gymnodinium catenatum*, responsible for the main PSP events along the Portuguese coast, and the potentially toxic species *Lingulodinium polyedrum* were recorded, but never accounted for significant relative abundances during the study period. Correlation between cyst assemblages and several environmental parameters were studied by correspondence analysis. Mean SST and surface Chl a were found to be important factors influencing cyst seasonality.

PO.01-08
Genetic variation in ribosomal DNA of *Chattonella* aff. *verruculosa*, a new harmful dictyochophyte forming recurrent blooms in Scandinavian waters

Session: PO.01 - Genetics

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The ichthyotoxic dictyochophyte initially named *Chattonella* aff. *verruculosa*, was for the first time recorded in Norwegian waters in 1998 and has since then formed recurrent blooms in the North Sea and Skagerrak. We have isolated strains from Skagerrak (2001 and 2006) and generated ribosomal DNA sequences from these strains, as well as of strains of *C. verruculosa* from other geographical regions. The relationships among these strains and to some other dictyochophytes were inferred from partial LSU (D1 and D2 domains) rDNA phylogeny. The *C.* aff. *verruculosa*/*C. verruculosa* strains diverged into two well supported clades: one clade included five *C*. aff. *verruculosa* strains from Skagerrak and the other embraced *C. verruculosa* from Germany, Japan and New Zealand. The distance between these clades was 1-2 % that may suggest a separation at the species level. The partial LSU rDNA phylogeny revealed *Florenciella parvula* as the closest relative to *C*. aff. *verruculosa*/*C. verruculosa*. We also amplified the ITS1- ITS2 ribosomal DNA region by PCR. The PCR products were cloned, and 5 clones from each of five *Chattonella* aff. *verruculosa* strains and *C. verruculosa* from Japan were sequenced. The ITS sequences showed intraspecific and intraclonal variation and was not related to geographical origin.

PO.13-52
First record of a harmful bloom of *Gymnodinium catenatum* along the Michoacán coast, México

Session: PO.13 - Regional events

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In Lazaro Cardenas, Michoacan in November 2005 the first record was made of a harmful algal bloom caused by the naked dinoflagellate *Gymnodinium catenatum* Graham 1943. *Cochlodinium polykrikoides* Margalef 1961 was also present in the bloom. *In situ*, *G. catenatum* formed chains of 12-64 cells, mostly 56 cells, while *C. polykrikoides* formed two-celled colonies only. The density of *G. catenatum* was 560,000 cells/liter compared to 20,000 of *C. polykrikoides.* The former species was the dominant dinoflagellate. Isolates of these two PSP producers are maintained as clonal cultures, not axenic, and maintained in a new medium

(L1VASE), a modification of the L1 media.

PO.10-50
Inorganic carbon acquistion in three red-tide dinoflagellates

Session: PO.10 – Ecophysiology and autecology

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Carbon acquisition was investigated in marine bloom-forming dinoflagellates, *Prorocentrum minimum*, *Heterocapsa triquetra*, and *Ceratium lineatum*. Activities of carbonic anhydrase (CA), photosynthetic O_2 evolution, CO_2 and HCO_3^- uptake rates were measured by membrane inlet mass spectrometry in cells acclimated to low and high pH. A second approach used short-term 14C-disequilibrium incubations to estimate the carbon source utilized by the cells. All species showed negligible extracellular CA activity in cells acclimated to low pH and only slightly higher activities when acclimated to high pH. Half saturation concentrations for photosynthetic O_2 evolution were low compared to RubisCO kinetics. Moreover, affinities for inorganic carbon increased with increasing pH in the acclimation, indicating the operation of an efficient CO_2 concentrating mechanism. Rates of HCO_3^- uptake were high in the investigated species, contributing more than 80% of photosynthetic carbon fixation. Affinity for HCO_3^- and maximum uptake rates increased under higher pH. Modes of carbon acquisition are consistent with the 13C-fractionation pattern indicating a strong species-specific difference in leakage. Our results suggest that photosynthesis in marine dinoflagellates is not limited by inorganic carbon even at high pH.

PO.05-12
Alternative bioassays for the detection of cyanotoxins

Session: PO.05 - Toxin analysis

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The principal method for detecting the presence of unknown cyanotoxins in Australia has traditionally been the mouse bioassay. When specific cyanotoxins are suspected, instrumental methods are generally used, but the identity of potential cyanotoxins is not always known and there is still the requirement for a broad screening assay for use in the water industry. Despite the widespread use and current reliance on the mouse bioassay, this test has several constraints. These constraints include: lack of precise quantification of cyanotoxins at low concentrations, insensitivity, difficulties in the interpretation of results and slow turnaround time. Additionally, animal ethics guidelines are calling for the elimination of the use of vertebrates in applications such as toxicity testing. This means that alternative bioassays for the detection of cyanotoxins in water supplies need

to be developed. Thus, the Combined Research Centre for Water Quality and Treatment has provided funding for this PhD study to investigate, optimise and validate of a range of microbial and invertebrate bioassays for cyanotoxin testing. The results relating to the sensitivity and applicability of these bioassays will be presented.

PO.05-21
Large-scale pumping and recovery of algal toxins from sea water

Session: PO.05 - Toxin analysis

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There is an expanding need for standard specimens of algal toxins for chemical analysis and toxicity testing. DSP toxins and azaspiracids, which are produced by non-cultivable algal species, can currently only be harvested from contaminated shellfish or directly from the alga when a bloom can be identified. We have developed a portable pumping system to harvest algae and lipophilic algal toxins directly from sea water. The automated system can filter 600-1000 L of water per hour and be run unattended for 3-4 days. The system has been evaluated with great success in Norway and Spain during the 2005–2006 season with tens of milligrams of DTXs and PTXs being harvested. The material obtained from this system is much easier to purify than material extracted from shellfish, and provides a convenient method for isolation and purification of algal toxin standards.

PO.07-10
A red tide incubator in the upwelling shadow of Monterey Bay, California

Session: PO.07 – Ecology and Oceanography

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Red tide blooms occur in coastal waters throughout the world. These blooms can harm ecosystem and human health, and create economic hardship. Due to limited understanding of red tide organisms and the environmental conditions leading to blooms, detection and monitoring of these events is challenging. In Monterey Bay, California, multi-platform remote sensing observations between 2002-2005 indicate the existence of a red tide 'incubator' in an upwelling shadow in a northern region of the bay. Relative to the rest of the bay, wind mixing in this region is weak, stratification is strong, and residence times are long. Additionally, this region receives input from estuarine waters, which influences stratification, nutrient input, and plankton species composition. Using high-resolution airborne remote sensing from PHILLS, AVIRIS and MAS, and time series of satellite observations from SeaWiFS, MODIS and MERIS, we detail characteristics of this incubator region. We integrate remote sensing with observations from moorings, ships, and

autonomous underwater vehicles, to show that the incubator region can seed bay-wide red tide events when mesoscale dynamics of the California Current stir the bay.

PO.13-73
Temporal and spatial distribution of the dinoflagellate genus *Alexandrium* along the Catalan coast (NW Mediterranean)

Session: PO.13 - Regional events

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The genus *Alexandrium* contains species associated with paralytic shellfish toxins. These species are morphologically similar and can be identified by small differences in the thecal plates using epifluorescence microscopy. Blooms and mixed populations of different *Alexandrium* species (toxic and non-toxic) are frequently observed at inshore areas of the NW Mediterranean Sea. Until now, ten *Alexandrium* species have been identified in this area, either in the water column or in the sediment, which corresponds to one third of the species described for this genus. We present the temporal and spatial distribution of seven *Alexandrium* species (*A. minutum*, *A. catenella*, *A. taylori*, *A. pseudogonyaulax*, *A. tamutum*, *A. insuetum*, *A. peruvianum*) based on samples collected during six years (2000 to 2005) on surface water of several harbours and beaches. Cell concentrations of the mentioned species are presented together with data of the relevant physical and chemical variables.

PO.15-08
The use of SPATT. Detection of Aza and comparison with toxin profiles in shellfish in relation to algal cell counts

Session: PO.15 - Monitoring

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We have developed solid phase toxin tracking (SPATT) disks based on the method of MacKenzie *et al*. (2004). Data from spatial and time-integrated sampling of disks with subsequent extraction and MRM LC/MS analysis for AZAs, PTX-2s, OA/DTXs, and YTX are presented. Profiles of toxins in the disks and blue mussels (*M. edulis*) are compared with the abundance of toxin producing algal species. Toxins accumulated in the disks and, generally, their concentrations followed the abundance of the algal species from which they had originated. However, large variations in toxin profiles were observed during mixed blooms of *Dinophysis* spp. At Flødevigen, mussels and disks contained substantial levels of DTX-2 throughout the trial; at Sogndal, only trace amounts could be detected in the disks from and DTX-2 could not be detected in the mussels. In mussels, AZA-1, -2, -3 and -6 typically occurred in ratios of ca 2:1:1:1, respectively. However, in extracts from the disks only AZA-1

and -2 (5:1) were detected, suggesting that AZA-3 and -6 may be produced by in vivo biotransformation in shellfish. SPATT disks are a convenient tool for monitoring toxigenic algal blooms, and should also be useful for identifying toxigenic algal species and studying toxin metabolism.

PO.05-17
Microcystins in the NIES Certified Reference Materials No. 26

Session: PO.05 - Toxin analysis

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The Certified Reference Material (CRM) for metal elements and microcystin analyses was prepared by NIES. The microcystins in the CRM are mainly [Dha7]microcystin-RR and -LR. To determine microcystin content in the CRM, we looked up authorized standards of [Dha7]microcystin variants. However, we could not obtain the standards. The microcystin content in the CRM was therefore determined according to the MMPB method. In the LC-MS analysis of the CRM, the results suggested the existence of [Dha7]microcystin-YR, [D-Asp3, Dha7]microcystin-RR and [D-Asp3, Dha7]microcystin-LR as the previously identified variants, and some unknown microcystins as minor variants. The NMR and MS spectra of the minor variants were measured for the elucidation of the structures. Amino acid compositions of the variants were also analyzed. All the data show that the minor variants are novel microcystin variants. The CRM is useful not only as standard material for quantification of microcystins but also for identification of [Dha7]microcystin variants. The CRM No. 26 with analytical data of microcystin variants has been supplied around the world.

PO.01-04
Development of microsatellite markers to study the population genetics of *Skelotonema* sp. - a marine diatom

Session: PO.01 - Genetics

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Skelotonema is a marine diatom species that contributes significantly to phytoplankton blooms. Resting stages are abundant in sediments. Allozyme studies have shown genetic differences between spring and autumn populations of *Skeletonema*. Recent studies have shown that phytoplankton can have extensive intraspecific variation within a geographic location. This variation can be measured using genetic markers such as RAPD and microsatellite DNA. These techniques can be applied to within-species analyses of *Skelotonema* resting stages and planktonic cells to study population differences. Microsatellites have never been used to discriminate between planktonic and benthic life stages of microalgal species. In the present study, *Skeletonema* genomic DNA libraries were constructed by microsatellite methods, and sequence data of microsatellite positive clones were obtained for PCR primer development. We have located (CA)n and (AT)n core sequences within the genome. Primer sequences flanking these tandem repeat regions are developed using the analytical software programs.

PO.15-21
Harmful algal blooms monitoring plan in the Chubut coastal waters, Patagonia, Argentina

Session: PO.15 - Monitoring

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The Harmful Algal Blooms Monitoring Plan design and implementation along Chubut coastal waters (Patagonia, Argentina) is reported. The provincial littoral zone involves more than 1000 kilometers with bays and gulfs where culture activities and shellfish harvesting from natural banks are carried out.
The plan includes five programmes: 1) Harmful algal blooms and water quality monitoring, 2) Shellfish toxicity control, 3) Educational and training activities and public information, 4) Summary of human consumers intoxication cases in Medical Centres, 5) temporal closing of shellfish fishing and selling.
This work communicates the results concerning the harmful algal blooms and environmental marine conditions monitoring, the activities of education and public information, the evaluation of results, the management plans and regulatory actions.
Toxic species observed in these coastal waters are: *Alexandrium tamarense*, *Prorocentrum lima*, *P. minimum*, several toxigenic species of *Dinophysis* and the *Pseudo-nitzschia seriata/delicatissima* complex. Temporal and spatial variations in relation to environmental parameters are analyzed.
Obtained data provide bases for public health protection and resources management actions. Educational activities involve training of the main actors as well as public awareness and communication.

PO.16-15
Abundance variations in dinoflagellate sedimentary cysts: the importance of time in sample analysis

Session: PO.16 – Life cycles

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Counting of dinoflagellate sedimentary cysts is time-consuming; thus, preservation methods are necessary to keep cysts intact until they are analysed. This experiment aims to establish whether the time of cold and dark storage and fixation methods affect the abundance of resting cysts in sediment samples.
To verify whether cyst abundance and species composition change with time, sediment samples from different sites (Sardinia, Italy; Catalan coast, Spain) have been studied. Subsamples were fixed with 10% formaldehyde before being treated with SPT (Sodium Polytungstate) at time zero (just after the sampling). The results

were compared to those from alive-fresh subsamples analysed at the same time. In addition, live subsamples were kept in the dark at two different temperature conditions (at 15 ºC and 4 °C); they were analysed one and two months after the samplings.
The results have provided information about which storage and/or fixation methods to use if cysts from sediment samples have to be stored for longer periods.

PO.05-36
Determination of paralytic shellfish toxins in seafood

Session: PO.05 - Toxin analysis

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Paralytic Shellfish Poisoning (PSP) represents a real threat to the health of shellfish consumers. The PSP toxins (saxitoxin group) are a group of naturally occurring neurotoxins that specifically block the excitation current in nerve and muscle cells by blocking the sodium channels. This can lead to paralysis if a large enough dose is consumed. The HPLC-FLD method based on Oshima, 1995, is currently in use at the Norwegian School of Veterinary Science for the analysis of PSP toxins. However, this method has been shown to be susceptible to inference peaks from a variety of different seafood matrixes. The poster will outline an improved SPE (solid phase extraction) based clean-up procedure for the complete removal of major chromatographic inference peaks. An improved extraction technique will also be presented.

PO.14-01
Potential role of clay in mitigating Chesapeake Bay algal blooms

Session: PO.14 - Mitigation

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Because of increasing concern for algal blooms in Chesapeake Bay and proximal coastal bays, laboratory studies were undertaken to examine the removal of several bloom species through the addition of treated kaolin clay. *Prorocentrum minimum*, *Chattonella subsalsa*, and a small coccoid cyanobacterium were grown in the laboratory and exposed to 0.9 g clay L^{-1}. In vivo fluorescence (IVF) was measured on 4 replicates for each taxon (control and treated) before clay additions, 2.5 h after clay addition, and 4 d later. There was a significant decrease in IVF in all clay treatments with largest reductions in IVF noted for *Prorocentrum* and *Chattonella* (99% and 92%, respectively) within 2.5 h of the addition; there was no further decline in IVF over the next four days. For the cyanobacterium, clay was not as effective, removing only 61% and 38% of total cells over the 4 d when initial densities were 10^9 and 10^8 cells L^{-1}, respectively. These results suggest that treated kaolin may be an effective mitigation strategy for flagellates common to Bay blooms whereas coccoid cyanobacteria may persist following clay additions.

PO.13-11
Crassostrea ariakensis and *C. virginica* responses to ichthyotoxic *Karlodinium veneficum*

Session: PO.13 - Regional events

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The Eastern oyster *Crassostrea* virginica and the Asian oyster *C. ariakensis* are native and potentially introduced oysters, respectively, in the Chesapeake Bay and as such, will be exposed to the natural phytoplankton assemblages including harmful species throughout their life cycles. Recent work suggests that at least one of these prey items, the ichthyotoxic dinoflagellate *Karlodinium veneficum*, occurs frequently throughout the growth period for the oysters and produces linear polyketide toxins (karlotoxins) which elicit toxicity through sterol-dependent, non-specific membrane pores. Spat and juvenile oysters of each species were exposed to moderately toxic strains [18.5 ± 6.2 ng ml^{-1}] at environmentally-relevant cell densities and growth and clearance rates contrasted with rates observed on other phytoplankton species, including the spring bloom former *Prorocentrum minimum* and phytoplankton mixtures routinely used in oyster hatcheries. Spat growth and clearance rates and juvenile clearance rates of both oysters were significantly reduced when feeding on *Karlodinium*, relative to the other prey species. These initial results suggest that the cosmopolitan *Karlodinium veneficum* inhibits growth and would potentially curtail oyster production within the tidal bay and its tributaries throughout the oysters' growth periods and considering its global distribution, potential impact throughout temperate areas should be assessed.

PO.13-89
The present state of toxin producing cyanobacteia species in the southern Lake Victoria (Tanzania)

Session: PO.13 – Regional events

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Cyanobacterial blooms have become an increasing worldwide problem in aquatic habitats. These occurrences can be partially attributed to gradual eutrophication of waterways caused by increase of nutrient inputs. Certain species of cyanobacteria produce toxins and, as a result, blooms create major threats to animal and human health, tourism, recreation, and aquaculture. Despite these facts, studies on toxic cyanobacteria species and their associated environmental factors are limited in the tropics. Worse still, in Eastern Africa, farmers and the general public are not aware of the challenges posed by toxin-producing cyanobacteria. In this paper, we present the recent status of toxin- producing cyanobacteria in relation to environmental factors linked to their dominance in Lake Victoria, from 2000 to 2005. The study revealed that blooms of cynobacteria occur frequently, and microscopic examination of the blooms showed dominance of the potentially toxic genera of *Microcystis, Anabaena* and *Cylidrospermopsis.* Average total abundance and biomass of phytoplankton were approximately 5 times higher than in the studies from the 1960s and 1980s. The persistence of toxic cyanobacteria appears to be favoured by a multiplicity of factors of importance: nutrient concentration (P), water temperature, mixing conditions, under-water light availability and food web structure.

PO.14-06
The use of clays to control harmful algal blooms in the U.S.: from laboratory to the field

Session: PO.14 - Mitigation

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For nearly a decade, the efficacy of clay flocculation and the potential impacts of clay dispersal have been examined in the United States. To date, the removal ability of various clays has been tested against *Karenia brevis*, *Heterosigma akashiwo*, *Prymnesium parvum, Aureococcus anophagefferens*, *Pfiesteria piscicida* and *Alexandrium tamarense*, with most of the research focusing on *K. brevis* and phosphatic clays. This presentation will summarize the major finding of the research from small to mesoscale studies – with and without flow – culminating in a pilot experiment in open waters during an actual bloom. Recent observations regarding the effect of phosphatic clays on the motility and viability *K. brevis* in flow will be presented. The potential benefits and disadvantages of using chemical flocculants such as polyaluminum chloride and cationic polymers in combination with clays will also be discussed. Finally, results from impact studies on select benthic organisms and several bivalve species, will be presented. Overall, this research has demonstrated the applicability of clays to treat harmful algal blooms under certain conditions in certain locations. However, there are also limitations to the method and possible impacts that must be considered.

PO.06-20
Microscopic digital holography imaging of dinoflagellate behaviour in laboratory cultures

Session: PO.06 - Population dynamics

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Predator-prey interactions are fundamental to a greater understanding of harmful algal bloom (HAB) dynamics; however, interactions between dinoflagellates

and their prey are difficult to monitor using standard microscopy. The high magnification needed to distinguish between HAB and prey species results in shallow depths of field and prevents tracking the 3-dimensional paths of multiple swimming organisms. Microscopic digital holography overcomes this limitation by using numerical reconstruction to provide in-focus views of all the organisms within a 3 mm depth. The accuracy in the tracking procedure is sufficient to provide fully 3-dimensional trajectories from one view. The technique is applied to cultures of toxic and nontoxic strains of *Karlodinium veneficum* with and without a predator, *Oxyrrhis marina*, as well as *Pfiesteria piscicida* with and without its algal prey, *Rhodomonas*. Typical swimming behaviors include helical swimming and conspecifics revolving around each other. *Karlodinium* individuals are observed to have swimming speeds ranging from 0.05 to 0.5 mm/s and *Pfiesteria* cells 0.1-1 mm/s. We also observe a change in the average swimming speed of *P. piscicida* from 0.5 mm/s in isolation to 0.9 mm/s in the presence of its prey, *Rhodomonas*.

PO.16-06
Effects of temperature and light on benthic cell germination and germinated cell survival of the noxious raphidophyte *Heterosigma akashiwo*

Session: PO.16 – Life cycles

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The effects of temperature and light on germination of benthic cells and survival of the germinated motile cells of *Heterosigma akashiwo* were examined with bottom sediments including the benthic cells of this organism. The motile cells appeared in a temperature range from 5 °C to 30 °C in suspensions of mixed sediment and seawater within three weeks, but at temperatures ≦12 °C, the cell numbers were markedly low and the appearance was delayed. When the samples, incubated at each temperature, were incubatedat 20 °C, only few motile cells appeared. In suspensions incubated in the light or dark, despite counting motile cells at six-hourly intervals, the number of motile cells germinated in the dark was significantly lower than those in the light. Subsequently, when the suspension incubated in the dark was exposed to light, only a few newly germinated motile cells were observed. These results indicate that germination of the benthic cells is independent of temperature and light if the sediment is suspended into seawater, but the speed of the germination process depends on temperature, and survival of the cells just after germination is strongly affected by temperature and light.

PO.15-27
Development of a simple and sensitive monitoring method for the shellfish-killing dinoflagellate *Heterocapsa circularisquama* using real-time PCR assay

Session: PO.15 - Monitoring

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Heterocapsa circularisquama is the most noxious red tide dinoflagellate along the Japanese coasts, causing mass mortalities of both natural and cultured bivalves. It is indispensable for mitigating the negative impacts to monitor this species rapidly, easily, and sensitively. Real-time PCR assay is a sensitive and specific method for the detection and quantification of microalgae, however, procedures for real-time PCR assay are not practical for field monitoring. In this study, we developed a simple and sensitive monitoring method for *H. circularisquama* using real-time PCR assay. Quantitative DNA extraction was made by boiling the filter in TE (Tris-HCl and EDTA) buffer after concentrating the cells on the Nuclepore filter. This assay made specific and quantitative detection possible even with the abundant presence of other algae. Enumeration of cells in natural samples revealed identical results by real-time PCR assay and the indirect fluorescent antibody technique even at cell densities as low as 1 cell per liter. Hence, this method is a powerful and feasible tool for monitoring of *H. circularisquama*. The work now continues using this method to understand population dynamics.

PO.01-03
Harmful algae can be transported via relocation of bivalve shellfish

Session: PO.01 - Genetics

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Our study tested the hypothesis that harmful algae can be introduced into new environments by means of shellfish relocations, a common practice for commercially-exploited bivalve molluscs. We identified which managed shellfish species and HABs co-occur geographically and established a protocol to assess the potential of the bivalve species to be vectors for transport of harmful algae. Cultured strains of harmful algae, *Alexandrium fundyense*, *Heterosigma akashiwo*, *Prorocentrum minimum*, and *Gymnodinium mikimotoi* were fed to bivalve molluscs for two days at a natural bloom concentration to assess the ability of the algal cells to pass intact through the digestive tract and subsequently grow. After feeding, the bivalves were kept for two days in ultrafiltered seawater. Biodeposits were collected and observed under the microscope after 24 and 48 h to evaluate the presence or absence of intact, viable cells or temporary cysts of the algae. Subsamples of biodeposits were transferred into both algal culture medium and filtered seawater and monitored microscopically for algal growth. Intact algal cells of the various harmful algae were seen in biodeposits and generally these re-established growing populations.

PO.10-42
Phosphatase activity in *Pfiesteria shumwayae*

Session: PO.10 - Ecophysiology & autecology

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Phosphatases, enzymes that hydrolyze organic phosphorus, include both alkaline and acid varieties with pH-dependent optima. Phytoplankton phosphatase research has focused primarily on alkaline phosphatase expression in photosynthetic species, including dinoflagellates. Acid phosphatases have been less studied in algae and have been examined in very few dinoflagellates (*Lingulodinium polyedrum*, Schmitter and Jurkiewicz 1981; *Crypthecodinium cohnii*, Barlow and Triemer 1986). Traditionally, phosphatase activity has been measured using colourimetric or fluorometric methods that cannot resolve variability within populations and among taxa in mixed assemblages. Recently, the molecular probe ELF-97® (Enzyme-Labelled Fluorescence; Molecular Probes, Inc., Eugene, OR) was developed for *in situ* fluorescence measurements of both alkaline and acid phosphatases in individual cells. In this study, ELF-97® was used to examine phosphatase activity in the heterotrophic dinoflagellate *Pfiesteria shumwayae*. Phosphatase activity also was evaluated at different pH values using colorimetric methods. Active phosphatases generally were localized in dense deposits near or surrounding the food vacuole. The location of enzyme activity and supporting colorimetric measurements suggest that acid phosphatases predominate in *P. shumwayae* and have a general catabolic function.

PO.13-22

Field and laboratory mortality and bloom decay rates of *Gymnodinium catenatum*: improving parameters in coastal models

Session: PO.13 - Regional events

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Phytoplankton mortality is rarely addressed in ecological models yet is seen by both modellers and ecologists as significant. Mortality rates of a number of micro-algal species present in the Huon Estuary Tasmania, Australia were assessed using both laboratory and field data. Particular emphasis was placed on *Gymnodinium catenatum*, a harmful dinoflagellate that forms recurrent blooms in the estuary. Mortality rates are new in marine ecological models so we transferred methodology and techniques from bacterial mortality models as these have been extensively researched because of food pathogens. Mortality rates for all microalgal species tested in the laboratory were between 0.04-0.3/day and field data for *G. catenatum* blooms from 1989 to 2004 concurred with these rates of decline. The mortality rates will be incorporated into the present biogeochemical models of the estuary and surrounding waters.

PO.10-12
Growth preferences and toxicity of *Chattonella* aff. *verruculosa* (Heterokontophyta)

Session: PO.10 - Ecophysiology & autecology

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The heterokont flagellate under the provisional name *Chattonella* aff. *verruculosa* has formed recurrent extensive blooms in the North Sea and Skagerrak since 1998. According to phylogenetic analyses *C*. aff. *verruculosa* belongs to Dictyochophyceae, not Raphidophyceae. Water samples were collected from Skagerrak from January to March 2006 and strains of *C*. aff. *verruculosa* were isolated. The cells were grown in IMR ½ medium with salinity 25 PSU and temperature 4 ºC. Twelve monoclonal strains were obtained using the capillary isolation method. The newly isolated strains and seven isolated from previous blooms in Skagerrak or elsewhere were grown under different temperatures, salinities and light climates to determine the optimal growth conditions. Based on field observations and culture experiments, *C*. aff. *verruculosa* seems to prefer cold, brackish water. The toxicity to various cell lines and fish preparations was also examined.

PO.13-83

Occurrence of phytoplankton potentially causing shellfish toxicity in the Skagerrak, the Kattegat and the Sound (Öresund) 1985-2005

Session: PO.13 - Regional events

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As part of the environmental monitoring programmes along the Swedish West coast phytoplankton composition and abundance has been monitored 1985-2005. Some of the data sets start in 1985 while others start ca 1990. Sampling is in general monthly and the Utermöhl sedimentation chamber technique is used for analyses of Lugol fixed samples. We report on the abundance of taxa and genera potentially causing shellfish toxicity. Genera include *Dinophysis*, *Alexandrium*, *Lingulodinium*, *Protoceratium*, *Protoperidinium* and *Pseudo-nitzschia*. Results indicate that phytoplankton producing DST (Diarrhetic Shellfish Toxins), PST (Paralytic Shellfish Toxins), AZT (Azaspiracidic Shellfish Toxins), Yessotoxins and Amnesic Shellfish Toxins (AST) occur regularly in the whole area. Comparisons with recommendations from the Swedish National Food Administration regarding regulatory limits for abundance of harmful algae are made. The long-term data sets and the seasonal distribution of the harmful phytoplankton species are presented. Also comparisons with long-term monitoring of DST in blue mussels (*Mytilus edulis*) along the Skagerrak coast is made.

PO.12-10

Parasites of the genus *Blastodinium* are peridinioid dinoflagellates

Session: PO.12 - Taxonomy and phylogeny

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Dinoflagellates of the genus i are parasites that spend part of their life cycle as multicellular trophonts inside the gut of marine, planktonic copepods. The individual cells in the trophont as well as the dinospores that they produce are thecate. The plate tabulation formula of *Blastodinium contortum* and *B. navicula* dinospores concurs with that of the order Peridiniales. In

phylogenetic analyses based on SSU rRNA genes, *Blastodinium* spp. branch with the typical, dinokaryote dinoflagellates. This taxonomic position of *Blastodinium* spp. is in contrast to current classifications in which the order Blastodiniales is thought to represent an early evolutionary branch of the dinokaryote lineage. Species currently included in Blastodiniales are all parasites, but there is a notable morphological diversity in this order. Molecular data does not suggest that members of Blastodiniales are monophyletic or even closely related and, therefore, the taxonomy of the group should be re-evaluated.

PO.04-04
Copepod grazing on a toxic *Dinophysis acuta* thin-layer bloom

Session: PO.04 – Food chains

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During a subsurface *Dinophysis acuta* thin layer bloom (max. 24000 cells.L^{-1}) off the NW coast of Portugal, we conducted a field evaluation of the importance of this dinoflagellate in the diet of 5 copepod species, covering 2 depth strata. The copepod community reached a maximum of 17,800 ind.m^{-3}, with an average of 8,000 ind. m^{-3}. The average presence of *D. acuta* in the copepods' digestive contents was low (0.3 cells.ind^{-1}) and related to the local dinoflagellate concentration. Only the larger copepods, *Calanus helgolandicus* and mostly *Centropages chierchiae*, showed relevant grazing, although restricted to locations with more than 9,000 cells.L^{-1} of *D. acuta*. On the contrary, the smaller species showed insignificant ingestion values, probably due to weak filtering capacities and/or to active rejection in the presence of other more edible phytoplankton. As these small copepod species accounted for 45% of all mesozooplankton, we can speculate that, despite the high potential grazing pressure, the thin layer bloom of *D. acuta* was not being top-down controlled. *Centropages chierchiae* showed the highest ingestion values (max. of 10 cells inside one individual), indicating some degree of 'active' feeding on *D. acuta* associated with its omnivore-raptorial behaviour and/or with a possible immunity to the toxins.

PO.08-23
Sodium chloride induces extracellular PSP toxin release from the cyanobacterium *Cylindrospermopsis raciborskii*

Session: PO.08 - Toxicology

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The filamentous cyanobacterium, *Cylindrospermopsis raciborskii* strain D9 from freshwater in Brazil produces PSP toxins - mainly saxitoxin (STX) and gonyautoxins (GTX2/3), and low amounts of dcSTX and dcGTX2/3. We analyzed the effect of NaCl concentration on growth and toxin production in strain

D9 in comparison with that reported for strain T3. Intra- and extra-cellular toxins were determined by post-column derivatization LC with fluorescence detection; structures were confirmed by LC-MS/MS. In contrast to strain T3, which is growth sensitive to NaCl (10mM), strain D9 grew at NaCl concentrations as high as 17 mM. At this high salt concentration, intracellular toxins levels in strain D9 cells were reduced by 80% and 95% for GTX2/3 and STX, respectively, by 36 h, concomitant with extracellular increase in GTX2/3. Decline in intracellular STX level was transient and returned to normal by 156 h. Extracellular toxin release was not associated with cell lysis because cell integrity determined by chlorophyll a content and cell morphology was maintained. These data suggest that D9 exhibits an active toxin release in response to high salinity. Regulation of PSP toxin synthesis and release appears to be differentially regulated by strains T3 and D9 under salt stress.
Fondecyt 1050433; Núcleo Milenio EMBA:PO4/007-F.

PO.11-13
Diatom effect on dinoflagellate growth

Session: PO.11 - Allelopathy

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Diatoms release secondary metabolites, but little is known about the ecological effect of these exudates. This study was conducted to test if diatom exudates have any effect on co-occurring dinoflagellates. For this purpose growth and primary production of: *Peridiniella catenata*, *Scrippsiella hangoei* and *Woloszynskia halophila* were determined in filtrates (0.2µm and 2.0µm) of diatom cultures and aged, autoclavated seawater. The growth of *P. catenata* and *W. halophila* was significantly reduced in filtrates of diatom cultures compared with aged seawater; *S. hangoei*, on the other hand, had increased growth rate in diatom filtrates. The response was generally greater in the 2.0µm filtrate compared with the 0.2µm filtrate. The instantaneous primary production was, however, not affected by the origin of the growth media. The results indicate that diatom exudates do affect the growth of the studied dinoflagellates. This effect is not due to immediate change in short term primary productivity but rather affects other growth regulating mechanisms.

PO.01-02
Dinoflagellate cysts from New Zealand ports and harbours, with emphasis on the distribution of harmful and potential invasive species

Session: PO.01 - Genetics

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Over the period 2003-2005, 179 sediment samples, collected from fourteen New Zealand ports and harbours, were analysed. The samples were part of a large-scale baseline survey for the NZ Ministry of Agriculture & Forestry, Biosecurity New Zealand. More than twenty distinct cyst types, representing nine dinoflagellate genera, were identified. The number of cyst types from individual ports and harbours varied from one to six, most belonging to the three common genera *Gonyaulax*, *Protoperidinium* and *Scrippsiella*. Six species were harmful: four were paralytic shellfish poison (PSP)-producers, *Gymnodinium catenatum*, *Alexandrium tamarense*, *A.* cf. *catenella* and *A. minutum*, and two potential yessotoxin/yessotoxin-like producers, *Protoceratium reticulatum* and *Lingulodinium polyedrum*. Cysts of *G. catenatum* were most widespread but confined mainly to the North Island ports and harbours. They coincided approximately with the sites of spread of *G. catenatum* blooms during the 2000 North Island PSP events. The possible spread of this species to new areas and potential invasive species from other countries are discussed.

PO.13-39
Domoic acid in Minke whale

Session: PO.13 - Regional events

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Just as human consumers of seafood contaminated with algal toxins are at risk of poisoning, other animals in marine food webs are impacted by these toxins. Documented cases of algal toxins associated with cetacean mortalities are limited; yet, recent research by scientists (USA and Canada) demonstrated that North Atlantic whales in the Bay of Fundy are exposed to PSP toxins. In Monterey Bay, domoic acid (DA), the ASP toxin, was reported in humpback and blue whale faeces following feeding on toxic planktivorous fish; demonstrating that algal toxins can be ingested and pass through the digestive tract of cetaceans. Recently, using HPLC and LC-MS, we detected DA (0.5 µg/g) in skin and blubber from a Minke whale stranded on the west coast of Scotland during a bloom of DA-producing diatoms (*Pseudo-nitzschia australis* and *P. seriata*). Little is known about how and to what extent algal toxins affect marine mammals. Toxin absorption across digestive tract membranes will play a major role in determining the dose interacting with the nervous system via the blood. Humans exposed to DA experienced nausea, vomiting, dizziness and confusion. If Minke whales are as sensitive as humans then they may also be likely victims.

PO.12-03
Diatoms from coastal environments of Buenos Aires Province (Argentina). Taxonomical analysis of genera that include species producing harmful algal blooms

Session: PO.12 - Taxonomy and Phylogeny

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Many diatoms are involved in HAB events. In addition to domoic acid-producing species, the literature reports cases of severe economic losses to aquaculture, fisheries and tourism by blooms of harmful non-toxic species. In the framework of a monitoring project in Buenos Aires coastal waters, we have detected several of these species. Phytoplankton was collected monthly at eight stations, with 30-µm net hauls, and examined with light and scanning electron microscopes. The aim of this study

is to analyze the specific diversity of *Coscinodiscus*, *Chaetoceros*, *Rhizosolenia*, *Thalassiosira*, *Cerataulina*, *Cylindrotheca*, *Leptocylindrus* and *Asterionellopsis*, to determine their temporal and spatial distribution, to solve taxonomical/nomenclatural problems and to detect blooms of the species reported as causing harmful events.
Seventy four taxa were determined. *Thalassiosira fryxelliae* is new to science, *Cerataulina dentata*, *Chaetoceros filiferus* and *Rhizosolenia hyalina* are new records for Argentina.
Among the potentially noxious species we found *Asterionellopsis glacialis*, *Cerataulina pelagica*, *Chaetoceros socialis*, *C. debilis*, *Coscinodiscus wailesii*, *C. concinnus*, *Cylindrotheca closterium*, *Rhizosolenia setigera*, *Thalassiosira curviseriata*, *T. mala*, *T. minima* and *T. minuscula*. Some of them were occasionally abundant, but only *Asterionellopsis glacialis* has been observed producing blooms that affected the tourism of the area during summer months.

PO.13-85
Recent reports on occurrence and toxin characterization of *Microcystis aeruginosa* – a freshwater toxic algal bloom from India

Session: PO.13 - Regional events

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In our routine fresh water algal floristic surveys, we have come across heavy algal water blooms of *Microcystis aeruginosa* in Cosmunda Pond, Champa district, and Kurung Tank, Bilaspur district, both in Chattisgarh State, Central India. The Cosmunda pond water bloom caused severe fish toll and human illness. No human deaths were recorded but heavy liver damage cases were reported. The pond is polluted by effluents from nearby cotton and silk textile industries. Kurung tank water is polluted with Jute industry effluents. Vishnoi *et al.* (2005) reported an abundance of *M. aeruginosa* in a water pond near Jodhpur, Rajastan, Western India, which was isolated and used for recovery of textile effluent. Studies related to the hepatotoxicity were associated with *Microcystis* blooms in Central India and reported by Ghosh *et al*. (2006). Jaiswal *et al.* (2006) reported on *Microcystis* strains and characterization of biocidal secondary metabolites. Kumar *et al*. (2006) surveyed several ponds of Varanas, North India, and found heavy infestation of *Microcystis* in several ponds. The toxin was idenfied by the mcyA gene segment.

PO.13-49
Diarrhetic shellfish toxin links to dinophysis populations in California coastal waters

Session: PO.13 - Regional events

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In Monterey Bay, California, *Dinophysis* has recently been implicated in the production of Diarrhetic Shellfish Poisoning (DSP) toxins. Our goal was to determine the annual *Dinophysis* cycle and possible relationship to DSP toxins in mussels. Collecting water and

mussel samples (*Mytilus californianus*) for 16 months at the Santa Cruz Wharf in Monterey Bay, we measured *Dinophysis* abundance in our lab and sent mussels to the regulatory Food Inspection Agency in Canada for toxin analyses. Results indicated that *Dinophysis* average abundance was higher (2,000 cells per Liter) in summer than in other months (90 cells per Liter) and DSP toxins in mussels were higher in summer (OA+DTX-1= 0.11 µg per gram) than in other months (OA+DTX-1= 0.04 µg per gram), though at least one of the DSP toxins was detectable nearly year round. A significant correlation between *Dinophysis fortii* cell biomass, the dominant biomass contributor, and OA concentrations in mussels suggests this species is the OA source, whereas the correlation coefficient became weaker when the biomass of other *Dinophysis* species was included. Thus DSP toxins, not previously monitored on the US west coast, may occasionally represent a potential health threat to human consumers of mussels, and possibly other filter feeding organisms.

PO.05-13
Preparation and simultaneous LC-MS analysis of fourteen shellfish toxins

Session: PO.05 - Toxin analysis

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For use as calibrants in LC-MS analysis, we prepared fourteen shellfish toxin standards: okadaic acid (OA), dinophysistoxin-1 (DTX1), pectenotoxin (PTX) -1, -2, -3, -6, azaspiracid (AZA) -1, -2, -3, yessotoxin (YTX), 45-OHYTX, brevetoxin-B2 (BTXB2), 7-O-palmitoylOA and 7-O-palmitoylDTX1. The former eleven toxins were purified from contaminated shellfish, except for YTX which was obtained from cultures of *Protoceratium reticulatum*. 7-O-PalmitoylOA and 7-O-palmitoylDTX1 were chemically prepared from OA and DTX1, respectively. The purity of the standard toxins was checked by 1H-NMR, LC-DAD, and LC-MS. All toxins were quantifiable in 30 min in a single run by monitoring negative ions, except for AZAs that were detected on positive ions. We proved the practicality of the method by carrying out recovery tests using 90% methanol extracts prepared from scallop and mussel hepatopancreas spiked with the standard toxins or extracts from naturally contaminated shellfish.

PO.13-30
Species dominance and permanence of *Gymnodinium catenatum* Graham blooms on the estern Mediterranean coast of Morocco (1994-2004)

Session: PO.13 - Regional events

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The chain-forming dinoflagellate *Gymnodinium catenatum* is the main species associated with paralytic shellfish poisoning (PSP) outbreaks in Moroccan coastal waters, since 1994. The seasonal distribution of *G. catenatum* in the Mediterranean coast of Morocco (35°05’N) during the period 1994-2004 is described. *Gymnodinium catenatum* is present throughout the year, but maximum concentrations are detected in spring (early May) and autumn (November). PSP toxicity was detected in shellfish after winter and autumn blooms. *Alexandrium minutum*, also a PSP agent, was observed in spring and summer. *Dinophysis caudata*, a DSP producer, bloomed occasionally in spring, and the potential ASP producer *Pseudo-nitzschia* spp. increased to bloom proportions sometimes in winter. A high correlation between toxic and non-toxic species has been established (r>0, 7); *Lauderia* spp. and *Thalassionema nitzschioides* with *G. catenatum*; *Ceratium* sp with *Dinophysis* spp; *Navicula* and *Ceratium karstenii* with *Alexandrium* spp; *Coscinodiscus* and *Leptocylindrus minimus* with *Pseudo-nitzschia* spp.

PO.05-16
On the correlation between MMPB and ELISA methods for total microcystin concentrations

Session: PO.05 - Toxin analysis

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Total microcystin concentrations of cultured cells and natural waterblooms were determined by the methods of MMPB and ELISA. In the case of ELISA, the cross-reactivity of the antibody and microcystin depended on the type of microcystin present. The cross-reactivity of microcystin-LR and RR was almost the same, whereas those of other variants were lower than that of microcystin-LR. When total microcystin concentrations of natural waterblooms were determined by the two methods, the results obtained from the MMPB method were always higher than those from the ELISA method. However, in some samples the results obtained from the ELISA method were higher than those from the MMPB method. These results suggest that some non-microcystin compounds reacted with the microcystin antibody, or the antibody was denatured by biosurfactants in the fractions of the cell extracts.
In the fractionation of natural waterblooms using HPLC, the cross-reactivity was found in non-microcystin fractionations. If the fractions did not contain even a trace amount of microcystin, non-microcystin compounds ought to have reacted with the antibody. To confirm this hypothesis, compounds in the non-microcystin fractions were isolated and purified by HPLC and thin-layer chromatography.

PO.14-13
Growth control of toxic microalgae cell by using direct current electricity, direct current high voltage electrical discharge, ozone gas dissolution and hydrogen peroxide

Session: PO.14 - Mitigation

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The direct current electricity is called DC (direct current), and has been used for portable apparatuses like batteries, flashlights, etc. The electric current in a solution shows electro-physical phenomena like the electrical short circuit through the surface. The DC high voltage discharge in water will find the

shortest route. On the other hand, the discharge will generate radicals in water and a bleaching effect can be expected. There are many ions such as nitric acids, phosphoric acid, calcium, magnesium present at the growth locality of algae, and the electric conductivity is therefore relatively good. By using ozone gas and hydrogen peroxide solution as references, the bleaching effect of the radicals on algae was examined.
Different varieties of algae were exposed to the DC high voltage discharge pulse and to the continuous DC. A bleaching effect was observed that seems to be caused by the radical induction in water and movement of algae to the electrode. A momentary temperature rise and temperature rise around the electrode, caused by the DC high voltage discharge, causes death of all algae regardless of the variety.

PO.09-06
First detection of azaspiracid outside European coastal waters

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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Outbreaks of DSP have been recorded in summer 2004 and 2005 at the same region of the north Atlantic coast of Morocco. DSP-positive samples of mussels detected by mouse bioassay were stored for further identification. Chemical analysis by HPLC/MS conducted in mussels harvested from Dar Hamra (DH) and Oulad Ghanem (OG) showed the presence of OA and DTX2. Analyses carried out in the same samples revealed also the presence of three main azaspiracid congeners, namely AZA1, AZA2 and AZA3. The discovery of the three azaspiracid congeners has been confirmed by mass spectra characteristic of each toxin.
Mussel samples harvested in 2004 in OG and DH, showed the toxin profile mainly composed of AZA2 (75 and 100%) and AZA1 (15 to 25%), but AZA3 was present at trace level. In 2005, the mussel toxin profiles from the same areas were slightly different and the relative composition was AZA2 (80 to 100%) and AZA1 (0 to 20%), and AZA3 at 10%. In both years AZA2 was three to four times higher than other congeners.

PO.07-03
Harmful algal blooms (HABs) in the South China Sea and their relations to marine and coastal environments

Session: PO.07 - Ecology and oceanography

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HABs occur frequently in the South China Sea (SCS), causing huge economic losses in recent years. This study analyzed historical HAB records for the period from 1980 to 2003, focusing on four major regions of the SCS: northern, southern, western and eastern regions. HAB affected area spread and its frequency increased, with

high frequent periods in 1990-1991, 1998 and 2002; the seasonal and annual variation, and the causative algal species of HAB were all different among the 4 regions. Many HABs occurred in the northern region, and two high frequent areas were off the Pearl River and the Manila Bay. Relatively, HABs occurred frequently during March-May in the northern region and in July in the western region. As causative species *Noctiluca scintillans* dominated in the northern region, and *Pyrodinium bahamense* in both eastern and southern regions. *Pyrodinium bahamense* and *Noctiluca scintillans* were the dominating species during 1980-2003, but more *Phaeocystis* cf *globosa* and *Alexandrium* sp blooms occurred 1991-2003. Those variations are related to regional ocean environments, such as reversed monsoon winds, river discharges, and upwelling, and also affected by local conditions, such as eutrophication off the Peal River and in the Manila Bay.

PO.13-42
Dynamics of harmful algal blooms in the Ukrainian coastal Black Sea

Session: PO.13 - Regional events

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During the last 10 years (1995–2005), 62 cases of microalgal blooms have been mentioned in the Ukrainian coastal Black Sea.
Basic main features of microalgal blooms in the Ukrainian Black Sea are:
– The number of microalgal species that produce blooms has increased (in the 1960s – 14, in 70-80s – 24, and in the 90s – 37 species);
– Blooms of *Skeletonema costatum* are frequent (15 cases) at all seasons, which indicate high levels of eutrophication. Maximal densities registered were 50 10^6 cells l^{-1};
– Within the last years, there has been a simultaneous dominance of 2-4 species of microalgae (*S. costatum*, *Heterocapsa triquetra* and *Eutreptia lanowii*);
– There has been a development of blooms caused by Chrysophyceae (*Emiliania huxleyi*, *Apedinella spinifera*) and Euglenophyceae (*Eutreptia lanowii*, *E. viridis*);
– Dinoflagellate species that did not cause blooms earlier (*Gymnodinium simplex*, *Gyrodinium instriatum*, *Scrippsiella trochoidea*) started occurring in this region;
– The density of the invasive toxic species *Cochlodinium polykrikoides* increased from 0,7-10^3 cells l^{-1} in 2001 up to 0,16-10^6 cells l^{-1} in 2005;
– Potentially toxic diatoms (*Pseudo-nitzschia seriata*, *P. delicatissima*) and dinoflagellates (*Gymnodinium aureolum*, *C. polykrikoides*, *Alexandrium pseudogonyaulax*, *A. tamarense*) are frequently dominant.
The analysis of long-term changes of blooms in the Ukrainian Black Sea has shown an increase in number of episodes from the end of the 90s.

PO.12-16
Does *Gambierdiscus toxicus* type material exist?

Session: PO.12 - Taxonomy and phylogeny

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Gambierdiscus species have a pan tropical distribution and some produce ciguatoxins which concentrate in marine food webs, causing ciguatera fish poisoning. Though *Gambierdiscus* cells are typically referred to as either *Gambierdiscus toxicus* or *Gambierdiscus* sp., the genus encompases a diverse multispecies complex. This species diversity is generally underappreciated in most ecological studies. Our research was undertaken to better characterize the *Gambierdiscus* species both morphologically, using SEM and calcofluor staining, and molecularly, using SSU through-D1-D3 LSU rDNA sequences. The molecular and morphological data support the existence of at least five new species, plus five of the six currently described species. The major problem was with *G. toxicus*, which could not be resolved because type material was unavailable. Also, despite an excellent description of the genus morphology in the original paper, the large range in cell sizes and multiple collection sites for the type material causes us to ask if the type *G. toxicus* description included multiple species. We would like to open discussions on whether type material exists or if a new, molecularly well defined, type species should be established. Once ecological studies include species level identifications, the differences in distribution, abundance and toxicity of CFP outbreaks may be better understood.

PO.13-68
A toxic benthic dinoflagellate ***Prorocentrum faustiae*** Morton isolated from Phanri Bay, South Central Vietnam

Session: PO.13 - Regional events

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Dinoflagellates were isolated from *Liagora sp*. (Rhodophyta) in a tidal area of Phanri Bay, Binhthuan Province. The species was identified as *Prorocentrum faustiae*. This is a new record of microalgae in Vietnam. The cells are widely oval, 30-40 µm wide and 40-45 µm long. Thecal surface is rugose with scattered large /and small pores except at the centre. A large nucleus and pyrenoid are positioned at the lower part and the centre of the cell, respectively. Intercalary bands are horizontally striated. This strain of *P. faustiae* produces two cytotoxic fractions, a non-polar and a polar fraction. The polar compound was found to be ichthyotoxic and the non-polar compound was found to be cytotoxic.

PO.13-40
Pseudo-nitzschia spp. and domoic acid in Maryland and Virginia waters

Session: PO.13 - Regional events

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Some species of the diatom genus *Pseudo-nitzschia* are known to produce the neurotoxin domoic acid (DA) which is responsible for amnesic shellfish poisoning in humans and domoic acid poisoning in animals. Current phytoplankton monitoring by Maryland Department of Natural Resources identifies *Pseudo-nitzschia* in water samples via light microscopy as either *P. seriata* or *P. pungens*. From 2002 to 2006 water samples were collected throughout the Chesapeake Bay (Maryland and Virginia), the coastal bays, and the Delaware coast for culture isolation, toxin analysis and *Pseudo-nitzschia* enumeration and species identification via TEM. Four species of *Pseudo-nitzschia* were identified in Maryland and Virginia waters (*P. multiseries*, *P. fraudulenta*, *P. pungens* and *P. calliantha*). *Pseudo-nitzschia multiseries*, *P. calliantha*, and *P. fraudulenta* have been isolated from field samples, cultured in the laboratory and sequenced (see poster by H. Bowers, this meeting). Domoic acid was found in 6 of 14 cultures and in some field samples containing *Pseudo-nitzschia*. *Pseudo-nitzschia* was most abundant in the southern portion of the Chesapeake Bay from January to March. The data suggest that *Pseudo-nitzschia* in the Chesapeake Bay region is more abundant at higher salinities and during colder months.

PO.10-44
Evidence for neurotoxins from species of the raphidophyte genera *Chattonella*, *Fibrocapsa* and *Heterosigma*

Session: PO.10 - Ecophysiology & autecology

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Blooms of raphidophyte flagellates were implicated with fish-killing events, intoxication of bivalves and allelopathic effects on other phytoplankton. The mode of toxicity is a complex one involving reactive oxygen species, hemolytic toxins, polyunsaturated fatty acids and neurotoxins. This last toxin category was inferred from HPLC separation of compounds that co-migrated with brevetoxins. Their actual structures and thus final identity remained to be defined. This study examined extracts of *Chattonella antiqua*, *C. marina, C. subsalsa*, *Fibrocapsa japonica* and *Heterosigma akashiwo*. Cultures of these organisms were grown in the laboratory to stationary phase, harvested, extracted with ethyl acetate. The dried residue was resuspended in absolute methanol, filtered and applied to the ELISA assay specific for brevetoxins and to ESI and MALDI HPLC Mass spectroscopy. All cultures were found to contain PbTX 2, -3 and -9. While the cellular concentrations of these toxins were low this evidence indicates that among the differing toxic substances produced in raphidophyte blooms, neurotoxins cannot be excluded as a contributor to their toxicity.

PO.15-15
Remote sensing for the detection and monitoring of *Microcystis aeruginosa* in western Lake Erie and Saginaw Bay, USA

Session: PO.15 - Monitoring

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The toxic cyanobacterium, *Microcystis aeruginosa*, has become a dominant component of the summer phytoplankton population in Saginaw Bay and western Lake Erie, USA. Expansive blooms of *Microcystis* have caused considerable concern to the Great Lakes region due to the use of these waters for recreation. The toxin, microcystin, has been observed in both regions above the recommended limit of 1 µg L^{-1} and poses a threat to human health. Microcystis blooms have unique scattering and absorption properties, due to the production of surface scum and the dominant accessory pigment, phycocyanin. In an effort to better detect and monitor these blooms, satellite derived products from SeaWiFS and MODIS which highlight these optical properties will be compared with in situ measurements of Microcystis and its toxin, from 2004 and 2005. Phycocyanin has an absorption peak centered at 620 nm. Therefore, changes in the absorption spectra centered at this wavelength will be investigated using spectral curvature methods. In addition, the use of a particulate backscatter ratio method will be tested for its ability to detect these blooms. The ability to detect surface scum using the MODIS near-infrared band (865 nm) may be compromised by shallow water depth but looks promising in deeper water.

PO.09-10
Fatty acid esters of pectenotoxin seco acids in Norwegian and Irish mussels

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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Pectenotoxin-2 (PTX-2) from marine dinoflagellates of the genus *Dinophysis* is rapidly hydrolyzed in many shellfish to pectenotoxin-2 seco acid (PTX-2 SA), which isomerises to 7- 7-epi-PTX-2 SA. Pectenotoxin-12 (PTX-12) from the same dinoflagellates is also believed to be hydrolyzed in shellfish to PTX-12 SAs, which are in equilibrium with isomers presumed to be 7-epi-PTX-12 SAs. Three series of fatty acid esters of PTX-2 SA and 7-epi-PTX-2 SA were detected by LC-MS analysis of an extract from Irish blue mussels (*Mytilus edulis*). Fatty acids were conjugated on C-11, C-33 and C-37 of the PTX-skeleton. In methanolic extracts from two samples of blue mussels from Norway, three series of fatty acid esters of PTX-2 SA, with only minor accompanying amounts of the 7-epi-analogues, and a series of fatty acid esters of PTX-12 SAs and its presumed 7-epi-analogues, were detected.
The location of the fatty acid ester linkages were identified by LC-MSn in positive- and negative-ionization modes, LC-MS analysis of the products of the reaction with sodium periodate, and NMR analysis of purified samples of the most abundant derivatives. For PTX-2

SA, the 37-O-acyl esters were the most abundant, while the most abundant side chain was the 16:0 fatty acid.

PO.14-12
Growth control of toxic microalgae by electrostatic adsorption and decentralization

Session: PO.14 - Mitigation

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Adsorption and desorption by static electricity happens here and there. An electrostatic phenomenon in the interface can be strong, and an unpleasant phenomenon caused by the static electricity in winter is accompanied with dry air. The influence of static electricity in water is not like in air, i.e. not caused by the electric charge. It can be observed in the interface between water and a body of an insulator and a semiconductor. Algae adhering to rice plants in a paddy field are a well-known phenomenon. It is also reported that straw bundles scattered in the paddy field adsorb algae. We thought that an electrostatic circuit formed in the environment around the rice and wheat straws rich in hydrated silica could possibly cause electrostatic adsorption and migration of algae.

The adsorption and desorption of algae around the straws of rice and wheat was studied by changing the electrostatic voltage and the current. The electrostatic induction in the environment is possibly influenced by the wind and the humidity around the straws above the water surface. An electrostatic induction model based on the straws as an induction medium was studied using toxic microalgae.

PO.06-12
A drifter study of a toxic *Pseudo-nitzschia* bloom from the Juan de Fuca Eddy in the Pacific Northwest

Session: PO.06 - Population dynamics

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As part of the ECOHAB-PNW program to study the community formation, domoic acid (DA) toxicity and transport of *Pseudo-nitzschia* from the coastal waters of Washington State and British Columbia to the shoreline, we employed drifters to follow blooms emitted from their putative origin, the Juan de Fuca Eddy. In September 2004, we followed the dynamics of a bloom of the highly toxic *Pseudo-nitzschia cuspidata* and a small, vertically migrating flagellate. Over ten days, particulate DA reached more than 40 nM and dissolved DA reached up to 15 nM. Our analysis of temporal changes allowed us to evaluate nutrient drawdown rates and corresponding biomass increase, and to test hypotheses regarding bloom success: 1) Is the bloom community composition a direct result of physical processes of eddy mixing of nutrients? 2) Is community composition the result of grazing

removal of competing species? 3) Is bloom formation a result of increased well-being of the successful phytoplankton species (measures of photosynthetic efficiency and potential)? We also tested the effect of both cellular and extracellular DA production on the competitive success of *Pseudo-nitzschia* (grazing reduction, improved cell physiology). This is one of the most comprehensive studies of an *in situ* toxigenic diatom bloom to date.

PO.10-49
Regulation of inorganic carbon acquisition in toxic diatoms under different pH

Session: PO.10 – Ecophysiology and autecology

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The effect of elevated pH on inorganic carbon acquisition was studied in three marine diatoms, two potentially toxic species *Pseudo-nitzschia multiseries* and *Nitzschia navis-varingica*, and the non-toxic *Coscinodiscus sp.*. In cells acclimated to low pH (7.9) and high pH (8.4 or 8.9), *in vivo* activities of extracellular carbonic anhydrase (eCA), photosynthetic O_2 evolution, CO_2 and HCO_3^- uptake rates were measured by membrane inlet mass spectrometry (MIMS). Independent of the pH in the acclimation, *P. multiseries* showed highest photosynthesis rates in comparison to the two other species. Large differences in the mode of carbon acquisition were monitored among species. While eCA activities were increased in *P. multiseries* and *Coscinodiscus* at elevated pH, *N. navis-varingica* showed low eCA activities independent of pH. Half-saturation concentrations ($K_{1/2}$) for photosynthetic O_2 evolution were highest for *Coscinodiscus* and lowest for *P. multiseries* and generally decreased with inceasing pH. CO_2 and HCO_3^- were taken up simultaneously by all species. $K_{1/2}$ for inorganic carbon uptake decreased with increasing pH with the exception of *N. navis-varingica*. The contribution of both carbon species to photosynthetic carbon fixation differed strongly among species. Whereas in *Coscinodiscus* HCO_3^- uptake contributed to carbon net fixation by almost 100%, it was around 60% in *P. multiseries*. In *N. navis-varingica,* it only accounted for ~ 30% indicating a strong preference for CO_2. Our results indicate strong species-specific differences in carbon acquisition among diatoms. In view of pH changes occuring especially during blooms as well as the ongoing acidification of the oceans, the observed differences in CCM efficiency and regulation may play an important role for the occurrence of diatoms.

PO.15-28
Use of geographic information system software and approaches to analyze long-term database

Session: PO.15 - Monitoring

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The historical Florida red tide database provides a cross-platform for applying and advancing GIS technology for the monitoring and visualization of HABs. By using the Temporal Analyst tool, the data are brought into an event which can be set to replay on any temporal scale; a HAB bloom can then be visualized and tracked over time. Tracking Analyst also creates animation loops in either a video (.avi) file or as an animated .giff. The Tracking Analyst Data Clock Wizard can be used to create a graph of concentric rings representing months of the year. The rings allow an illustration to be created that represents certain temporal patterns in the database. This is helpful in understanding when a event begins and ends. The data clock is able to show that bloom events are able to carry over from previous years. GIS technology is advancing rapidly to the point that seamless merging of different databases can occur almost near real-time. This has applications for monitoring programs involving platforms, e.g., buoys, gliders, satellites, etc. If the intent is to have web-based data management and visualization, then advancing GIS technology will further this approach. End products would be maps and animations for time series.

PO.01-11

Molecular approaches for the detection and characterization of *Alexandrium* species in natural blooms

Session: PO.01 - Genetics

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Various molecular methods were used to detect and discriminate *Alexandrium* species in environmental samples collected along the Scottish east coast in 2004. Plankton samples were collected by vertical plankton net (20 µm) hauls (20-0 m) as well as by Niskin bottle casts from discrete depths. Cells filtered onto polycarbonate membranes were analysed using fluorescence *in situ* hybridisation (FISH) in combination with solid-phase cytometry to detect and enumerate *Alexandrium tamarense*, *A. ostenfeldii* and *A. minutum*. Additionally, conventional and real-time quantitative PCR (RT q-PCR) was applied to the same field samples to detect, differentiate and quantify the different *Alexandrium* species. For this approach, new species-specific primers and MGB-probes targeting the small- or large-ribosomal subunit of *Alexandrium* spp. were developed and successfully applied in both PCR approaches. The obtained results were compared with Utermöhl microscopic counts and toxin profiles generated by HPLC-FD and LC-MS/MS to detect PSP toxins and spirolides, respectively. The combined data set was analyzed by multivariate statistical methods to describe the vertical distribution of toxic species and their respective toxins profiles.

PO.13-41

DSP toxins in the Gulf of Finland, Baltic Sea

Session: PO.13 - Regional events

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Dinophysis species are known to be toxic around the world. Their toxins accumulate in filter-feeding animals like mussels, and may cause diarrhetic shellfish poisoning to humans. In the Baltic Sea, there are only few measurements of these toxins in the *Dinophysis* cells. DSP toxins (DTX and PTX) have been found in Dinophysis cells on the Finnish coast in late summer and autumn 2004. Spatial variability of DSP toxins is measured along a coastal gradient in the Gulf of Finland. In addition, the DSP toxicity is related to the occurrence of *Dinophysis acuminata*, *D. norvegica* and *D. rotundata* and varying environmental conditions.

PO.09-05
Profiles of PSP toxins in shellfish from Portugal explained by decarbamoylase activity

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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The presence of PSP toxins has not been recorded along the Portuguese coast since 1995. A bloom of *Gymnodinium catenatum* occurred at the NW coast of Portugal in the autumn of 2005, and PSP profiles were determined in several inshore and offshore shellfish species by HPLC. Most of the species studied contained a complex toxin profile, typically representative of contamination by *G. catenatum*. However, species such as the clam *Spisula solida* contained mainly decarbamoyl toxins; carbamate and N-sulfocarbamoyl toxins were recorded only at trace levels. In vitro incubation of S. solida extracts with PSP standards revealed a rapid transformation of carbamate and N-sulfocarbamoyl toxins into their corresponding decarbamate analogues. After 24 hours less than 5% of the carbamate or N-sulfocarbamoyl toxins tested remained.

PO.08-21
Neuroblastoma cells as a model to study toxic events triggered by palytoxin

Session: PO.08 - Toxicology

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One of the most potent marine toxins related to seafood poisoning is palytoxin, a non-TPA-type skin tumour promoter. The presence of palytoxin has been detected in several fish, crabs and sea anemones, from where it can enter into the food chain and cause human fatalities. The receptor for palytoxin is the plasma membrane Na+/K+-ATPase, and it stimulates sodium influx and potassium efflux in every system where it has been tested. Altered intracellular cation concentrations, in particular increased calcium, are generally associated with cell death or apoptosis. However, biochemical signalling cascades that transmit palytoxin-stimulated signals remain to be clarified.
In vitro cytotoxic effects of palytoxin were characterized on the BE(2)-M17 human neuroblastoma cell line. By using microplate fluorimetric techniques, we studied several cytotoxicity features, including markers of cell death and apoptosis,

such as changes of mitochondrial membrane potential, inhibition of cellular proliferation, DNA fragmentation, LDH leakage, propidium iodide uptake, and F-actin depolymerization.
Results show that palytoxin causes strong F-actin disruption depending on concentration and incubation time. Palytoxin induces fast cell rounding, a fall in mitochondrial membrane potential that is dependent on the concentration and the time of incubation, release of LDH from the cells, and it inhibits cell proliferation.

PO.13-79

Occurrence of the toxic dinoflagellate *Prorocentrum lima* in the Caribbean coast of Costa Rica

Session: PO.13 - Regional events

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A monthly survey was carried out in five localities along the Caribbean coast of Costa Rica in 2005, to determine the presence and distribution of *P. lima*. This dinoflagellate, which is known to produce OA, was identified growing in association with macroalgae, which typically dominates the subtidal zone near areas of coral reef. *Prorocentrum lima* appeared to have a substrate preference in favour of *Sargassum* sp. and *Padina* sp. with more than 200 cells/g D.W. This dinoflagellate was present year round in all the survey spots, primarily in the southern region during March and September. This is the first report of macroalgae associated with *P. lima* in the Caribbean of Central America. *Prorocentrum lima* was isolated from macroalgal sediments, cultured and tested for toxicity with triplicate using the standard brine shrimp bioassay, each with 10 *Artemia* microalgal cultures (1000-500-250 cells/well) with a stationary phase of 24 hours. Toxicity was found in *P. lima* cultures, which killed seventy five percent of the *Artemia* during the first 10 h of the bioassay. Cultures of *P. micans* were used as non-toxic microalgal controls which did not affect the viability of *Artemia*. Further work will be carried out by HPLC to determinate the production of OA by these organisms.

PO.10-47

Importance of nitrogen and phosphorus availability on the regulation of *Prorocentrum lima* growth and okadaic acid production

Session: PO.10 - Ecophysiology & autecology

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The benthic dinoflagellate *Prorocentrum lima* has been associated with many diarrhetic shellfish poisoning (DSP) outbreaks worldwide. The major causative agent of DSP outbreaks in Greek coastal waters (E Mediterranean) is *Dinophysis acuminata,* an okadaic acid- (OA) producing species.

However, *D. acuminata* cannot be grown in cultures, while *P. lima* can, and therefore *P. lima* cultures are used widely in OA-related studies. In this study, a toxic *P. lima* strain was grown in batch cultures under: 1) N-NO_3^- sufficient, 2) N-NO_3^- deficient, 3) P-PO_4^{3-} deficient and 4) N-NH_4^+ sufficient conditions for 57 days. Cell numbers, growth rates, chlorophyll a, OA concentrations per cell and nutrient concentrations in the culture media were determined at regular intervals. Phosphorus was exhausted in the P-deficient and N-NO_3^- sufficient treatments after the end of the typical exponential phase. Nitrogen decreased but was not exhausted in any treatment. In all treatments, maximum OA concentrations per cell were observed after the end of the typical exponential phase, when growth rates decreased. However, the highest OA concentration in *P. lima* cells was found in the P-deficient treatment (2 times higher than any other treatment), probably due to further limitation of growth induced by the heavy deficiency of phosphorus.

PO.11-05

Talk to me – communication between cyanobacteria via toxins and promotion of oxidative stress

Session: PO.11 - Allelopathy

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Cyanobacteria are now common in many freshwater lakes and rivers throughout the world and are known to produce a variety of different, often toxic, secondary metabolites. The ecological use of these metabolites is still under discussion. One main idea on cyanotoxins might be the use as info chemicals between algal cells in the interaction of algae and macrophytes. This study investigates the idea that cyanobacterial toxins are a communication tool between different cyanobacteria. This communication might be an indirect one, as cyanotoxins might be a trigger for the promotion of oxidative stress, generating reactive oxygen species, which are known to act as cell signals. The existence of several antioxidative enzymes likes superoxide dismutase, peroxidases and catalases as markers for oxidative stress in *Synechocystis* sp. will be shown as well as cell damage by extensive generation of ROS like lipidperoxidation due to exposure of *Synechocystis* sp. to the cyanobacterial toxin microcystin-LR. As reactive oxygen species are also thought to have an important role in the communication in cells, the main hypothesis presented here, is that the communication between cyanobacterial species might be mediated by ROS and the antioxidative cycle.

PO.13-76

Space distribution of potentially harmful species on the coast of the state of São Paulo, Brazil (July/04-May/06)

Session: PO.13 - Regional events

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This study reports on the space distribution of potentially harmful species on the coast of the State of São Paulo, Brazil (July/04-May/06), based on a survey carried out in cooperation with the State Environmental Agency and mariculture farms to provide

information to protect tourism and safety for shellfish consumption. The study of whole water and net samples (20-μm mesh) revealed the presence of *Anaulus australis**, *Asterionellopsis glacialis**, *Cerataulina pelagica*, *Coscinodiscus wailesii*, *Guinardia delicatula*, *Leptocylindrus minimus*, *Pseudo-nitzschia* spp.*; *Ceratium fusus**, *C. hircus*, *Dinophysis acuminata*, *D. caudata*, *D. rotundata*, *D. tripos*, *Gymnodinium catenatum*, *Noctiluca scintillans*, *Peridinium quinquecorne*, *Prorocentrum micans*, *Trichodesmium thiebautii* and *Dictyocha fibula* (* species that reached 10^4 cells.L^{-1} and/or whose relative abundance was over 50% of microphytoplankton count). No blooms were visually observed during this period. *Anaulus australis* and *A. glacialis*, species that have caused surf blooms in the area, were indeed dominant in more exposed beaches. *Pseudo-nitzschia* spp., *L. minimus*, *C. fusus* and *P. quinquecorne* were some of the most abundant ones in more protected embayments, the environment where mariculture farms are located. Interestingly, the recently introduced species to Brazilian waters, *G. catenatum*, was found to be more restricted to the surroundings of a port terminal.
Support: Biota-Fapesp, Cetesb, Butantan, CNPq-PIBIC.

PO.01-13
Petroleum production platforms as sites for the expansion of ciguatera in the northwestern Gulf of Mexico

Session: PO.01 - Genetics

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There are records of ciguatera in the northwestern Gulf of Mexico, a region characterized by soft muddy bottoms considered to be poor habitat for the CTX source dinoflagellate *Gambierdiscus toxicus*. We examined petroleum production platforms and *Sargassum* as potential substrate for *G. toxicus* and report a first record of this species in the NW Gulf of Mexico. All platforms (n=6) and some of the *Sargassum* (n=3) examined harbored *G. toxicus*. Only minor toxicity (<0.15 ppb) was noted in 2 of 20 great barracuda (*Sphyraena barracuda*) examined. Trans-Gulf migrations by barracuda are common; thus, we cannot determine if the toxicity was acquired locally or transported in migrating fish.
The rapid increase in platforms since 1942 has provided a novel substrate for benthic dinoflagellates in an otherwise inhospitable environment, and the platforms serve as intersection points for potentially toxic fish and fishermen. The use of platforms as fisheries enhancement structures could have unintended consequences for human health, particularly if projected rising sea surface temperatures over the next century alter dinoflagellate distributions and fish migration patterns.These concerns may also extend to mariculture operations around rigs or wind-farms, both of which would also add substrate for epibenthic microalgae.

PO.01-19
Distribution and diversity of toxigenic *Microcystis* blooms: a temperate-tropical comparison

Session: PO.01 - Genetics

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Cyanobacterial blooms were investigated in shallow lakes and ponds in Belgium and microdams in Ethiopia during respectively three and two consecutive years. DGGE-profiling of 16S rDNA revealed that the most dominant bloom-forming genus was *Microcystis* in both countries. DGGE-profiling of *Microcystis*-specific16S-23S rDNA ITS revealed a significant diversity within and among sites. PCR was used to detect the presence of mcy-genes within blooms and revealed that most blooms contained toxigenic strains. Phylogenetic analysis of ITS-sequences was used to relate toxigenic capacity to the genetic composition of blooms. Multivariate analyses were used to relate bloom composition to climatic condition, nutrient status and biological characteristics.

PO.15-02
The potentially harmful algae and HABs in the East China Sea by regular red tide monitoring programme

Session: PO.15 - Monitoring

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About 435 red tides are recorded in the East China Sea since the 1930s, which amounts to 54% of total red tides in China. Among them, 43 toxic HABs were recorded; the causing species include *Alexandrium tamarense*, *Karenia mikimotoi*, *Heterocapsa circularisquama*, *Cochlodinium* sp. and *Karenia brevis*. The HABs of *Karenia mikimotoi* in 2005 caused direct loss of about RMB 20 million by killing cultured fish (red drum, genuine porgy) and abalone. There are about 24 recorded blooms of *Karenia* sp. in the East China Sea since 1980s, most of them have detrimental influence, and the bloom of *Karenia* sp. has a tendency to increase in frequency and to be spreading from south to north. According to regular phytoplankton monitoring since 2002, there are 19 potentially toxic algae: PSP-causing algae (*Alexandrium catenella*, *Alexandrium tamarense*); DSP-causing algae such as *Dinophysis caudata*, *Dinophysis fortii*, *Dinophysis acuminate*; NSP-causing algae, *Karenia brevis*, ASP causing algae such as *Pseudo-nitzschia multiseries*, *Pseudo-nitzschia multistriata*, *Pseudo-nitzschia delicatissima*, and other 8 toxic algae such as *Heterocapsa circularisquama*, *Cochlodinium* sp., *Fibrocapsa japonica* etc. A monitoring scheme of toxic algae and biotoxin is put forward.

PO.02-04
A proteomic approach to harmful algal bloom research

Session: PO.02 - Genomics

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Proteomics, defined as the study of proteins expressed by the whole genome at the setting time and conditions, has been applied in the studying of harmful algal bloom (HAB) research and has shown high potential for identification of species-specific proteins for species identification; toxin biomarkers for strain differentiation; physiological indicator proteins to rapidly assess the nutritional or metabolic status of causative agents of HAB for prediction, detection, monitoring and verification of bloom events; and cell surface associated proteins to study the interaction between phytoplankton and their environment as well as to separate species from mixed phytoplankton populations. The paper will present a review of the various techniques we applied; namely 2-DE, 2-D DIGE, MALDI TOF MS/MS, N-terminal sequencing and immunoblotting; and the results we have obtained in HAB studies.

PO.16-02
Distribution of dinoflagellate resting cysts in surface sediments from Changjiang Estuary in the spring of 2004

Session: PO.16 – Life cycles

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Surface sediments were sampled in April and May of 2004 from southern Changjiang Estuary, where a large- scale *Prorocentrum donghaiense* red tide accompanied by *Alexandrium* sp. occurred in early May of 2004, in order to study cyst composition and distribution before and during the red tide. Results showed only few differences in cyst composition between the two surveys. The average cyst concentrations in April and May were 374 and 482 cysts per gram D.W. sediment, respectively. Cysts of *Alexandrium* occurred commonly but in low numbers, and the maximum concentration during the bloom was 219 cysts per gram D.W. sediment. High sand content in sediments, high sedimentary rate, huge diluted water and complicated water currents, are thought to result in the low cyst assemblages in Changjiang Estuary. The destination of the *Alexandrium* cysts formed after the bloom is discussed as.

PO.06-10
The interspecific competition of two HAB species: *Prorocentrum donghaiense* and *Alexandrium tamarense*

Session: PO.06 - Population dynamics

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We studied the population dynamics and interspecific competition of *Prorocentrum donghaiense* and *Alexandrium tamarense*, two common HAB species in East China Sea, by using semi-continuous dilution experiments. *Prorocentrum donghaiense* was the winner at low phosphate concentrations, which completely excluded *Alexandrium tamarense*. However, at high phosphate conditions, the competition between the two species was different, and either caused exclusion of one species or coexistence. The results also show that the competition results depend on the cell densities which the two species can reach in bi-culturing experiments.

PO.07-15

The role of nutrients on spring and summer algal blooms in the East China Sea

Session: PO.07 - Ecology and oceanography

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Diatom and dinoflagellate blooms were investigated in the East China Sea 2002-2005. When diatom blooms started in spring, the phosphate concentration decreased from about 0.6 µM to 0.2 µM, which suggests that phosphate might have been the limiting factor for the diatom blooms. At the succeeding dinoflagellate bloom, the phosphate concentration decreased to 0-0.1 µM. *Prorocentrum donghaiense* Lu was capable of growing even at phosphate concentrations below 0.2 µM.
Nitrate concentration showed a gradual decrease, concomitant with the algal bloom development. At the end of *Prorocentrum donghaiense* bloom, the average nitrate concentration was about 1.5 µM in the surface water. We hypothesized that dinoflagellate blooms could not be maintained when nitrate concentrations were lower than the above-mentioned level.
It was concluded that phosphate concentrations are essential for triggering the blooms, nitrate concentrations determine the duration and scale of the bloom, and silicate concentration and Si/N ratios influence which species will be dominant. By determining nutrient concentrations, algal blooms in the East China Sea can be forecasted relatively accurately.

PO.15-22

Application of a Multiparameter Monitoring System (YSI) in studies of large-scale red tides in the East China Sea

Session: PO.15 - Monitoring

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The multiparameter monitoring system (from YSI), coupled with a global positioning system (GPS) and an automatic meteorological station, has been used in field cruises to study the large-scale red tides in the East China Sea, supported by the on-going CEOHAB project. Parameters including chl.a, temperature, salinity, turbidity, dissolved oxygen, pH, and depth were recorded with the YSI system during the cruises during the last five years. The results suggested that there were good correlations between the measured chl.a levels by the system and the analytical results of chl.a from fluorescence spectrometer and cell counting by microscopy, during the red tides of *Prorocentrum donghaiense*, *Karenia mikimotoi*, *Skeletonema costatum*, and *Thalassiosira sp*. The spatial and temporal distribution pattern of the red tides, even the subsurface thin layer distribution of high concentrated microalgae, could be depicted with the system. Data on chl.a, temperature, salinity and depth derived from the system were used successfully to elucidate the hydrological effects of water masses and ocean currents on the dynamics of large scale red tides.

Therefore, data from the YSI system could well support the studies on the dynamics and mechanism of large scale red tides in this region.

PO.12-05
Do you know this dinoflagellate?

Session: PO.12 - Taxonomy and phylogeny

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During routine ballast water monitoring for harmful algae we discovered an unusual dinoflagellate. The cyst was obtained by sediment sampling a ballast tank of the cargo container ship ‘Southern Fighter’, docked at the Port of Tampa. In culture, this dinoflagellate spends the majority of its life cycle in a non-motile stage. The non-motile stage is twice the size of the vegetative cell. It is smooth walled, with the exception of the apical pore area, and gathers in large clusters that are visible to the naked eye. The vegetative cell is a scrippsielloid dinoflagellate with the following plate tabulation: Po, x, 4’, 3a, 7', 6c, 6s, 5'', 2''. The vegetative cell is characterized by a deeply invaginated and narrow 1’ plate, unique apical pore complex, and deep sulcus. The vegetative cells have been morphometrically compared to *Bysmatrum*, *Calciodinellum*, *Ensiculifera*, *Pentapharsodinium*, *Peridiniella*, *Peridinium*, *Protoperidinium*, and *Scrippsiella* with no definitive match, although they most resemble *Bysmatrum*. We presume this dinoflagellate is a benthic species like *Bysmatrum*, but it lacks the anterior intercalary plate pattern typical of that genus. The routine ports of call for this ship, where ballast water exchanges took place, were Antwerp, Belgium, Terneuzen, Netherlands, and Uddevalla, Sweden.

PO.10-06
Interaction effects of nutrient limitation and UV radiation on *Nodularia spumigena* - an outdoor experiment

Session: PO.10 - Ecophysiology & Autecology

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Nodularia spumigena is one of several toxin-producing cyanobacteria in the Baltic Sea. It produces the hepatoxin nodularin, a tumour promoter known to have killed wild and domestic animals. *Nodularia spumigena* blooms occur during late summer, a period with strong light, calm weather and stable water-column stratification. Due to its ability to fix atmospheric nitrogen, *N. spumigena* does not depend on this macronutrient in the water. Instead it is generally believed that growth of *N. spumigena* is limited by phosphorus. In a mesocosm experiment we will study the interaction effects of N and P limitation, and UV radiation. Cultures of *N. spumigena* are exposed to, or shielded from, natural levels of UV radiation in 5-L aquaria with and without N- and P-limiting conditions, repectively. The experiment will be run for ca 30 days (July, 2006). Variables to be analysed are related to growth and photosynthetic activity as well as the content of nodularin and UV-absorbing compounds.

PO.07-14
Paralytic shellfish poisoning in the North Sea – a secular perspective

Session: PO.07 - Ecology and oceanography

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Paralytic shellfish poisoning (PSP) has been a recognized medical syndrome since the late eighteenth century, despite the rarity of diagnoses. European records from 1827 to 1967 (140 years) in which the clinical symptoms are unequivocal number only about 100 cases distributed between nine outbreaks, mostly in the United Kingdom (UK). Since 1968, following a major episode in the UK that year, monitoring programmes indicate that the risk of poisoning has been high most years, and that in the absence of public health initiatives would almost certainly have led to many more cases. Thus on the basis of its clinical incidence prior to 1968, and monitoring since then, an abrupt 'regime shift' has apparently occurred which has probably led to increased abundance of the toxic agent, *Alexandrium tamarense*, since the 1960s. Some recent studies indicate that there may have been significant phenological trends in North Sea dinoflagellates, but the clinical evidence does not unambiguously support a climatic interpretation in this case. This paper uses network analysis, in which fishing plays a major role, to explore the question.

PO.15-07
Moving towards an operational harmful algal bloom forecasting system in Texas (USA)

Session: PO.15 - Monitoring

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Blooms of the toxic harmful algae, *Karenia brevis*, have been persistent and problematic in the Gulf of Mexico (USA) for decades. A heuristic ecological model based on satellite imagery has been shown to be effective in identifying likely *Karenia* blooms in the eastern Gulf of Mexico (Florida). 'New' blooms are identified by satellite, and those blooms that meet certain criteria are identified as likely *Karenia*. The method is core to the detection component of the operational Harmful Algal Bloom (HAB) forecast deployed by the National Oceanic and Atmospheric Administration (NOAA), in October 2004. The Texas coast commonly has false positives resulting from frequent resuspension events. The method was modified to compensate for these events. However, because of the infrequency of *Karenia* HABs along the Texas coast, only one major bloom event coincided with available SeaWiFS imagery. This event was used to validate the methods presented by Wynne *et al.*

(2005). Since the publication of this paper the Texas coastline experienced a major bloom in 2005. This presentation will demonstrate the efficacy of the algorithm using in situ cell count data collected from this event. This algorithm will then be used to expand NOAA's HAB forecast system to include Texas.

PO.10-48

Intracellular phosphorus regulates alkaline phosphatase activity of *Karenia mikimotoi* (Dinophyceae) and *Skeletonema costatum* (Bacillariophyceae)

Session: PO.10 – Ecophysiology and autecology

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Alkaline phosphatase (AP) plays an important role in utilization of organic phosphorus by unicellular algae. AP activity has been recognized to be regulated by the external phosphate concentration. In the present study, however, we clearly showed that AP activity of harmful algae *Karenia mikimotoi* and *Skeletonema costatum* was directly regulated by the intracellular phosphorus content, not by the external phosphate. Under phosphorus-limited steady state conditions in semi-continuous culture, relationships among AP activity, external phosphate and intracellular phosphorus content (Q) were examined. A significant negative correlation was found between AP activity and Q of *K. mikimotoi*. In the case of *S. costatum*, AP activity increased with increasing Q and reached a maximum of ca. 4 fmol/cell, then the activity decreased with increasing the Q. The threshold values of Q for inducing AP activities in both species were much higher than the minimum cell quota for their growth. The results indicate that the intracellular phosphorus concentration is fundamentally important to understand AP regulation and organic phosphorus utilization of harmful algae.

PO.11-09

Allelopathic interactions between the bacillariophyte *Skeletonema costatum* (Greville) Cleve and the raphidophyte *Heterosigma akashiwo* (Hada) Hada *ex* Hara *et* Chihara

Session: PO.11 - Allelopathy

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Growth interactions between *Skeletonema costatum* and *Heterosigma akashiwo* were investigated by bi-algal cultures under axenic conditions. When these species were inoculated at high cell densities, growth of both species was coincidentally suppressed. In other combinations of inoculation density, the species that first reached the stationary phase seemed to be responsible for a remarkable decrease of the other species' density. When both species were cultured without cell contact, growth of *S. costatum* and *H. akashiwo* were both suppressed. Furthermore, regardless of nutrient re-enrichment, filtrates from *S. costatum* and *H. akashiwo* thick cultures mutually decreased the maximum cell densities. Therefore,

growth interactions strongly suggest the involvement of allelopathic substances secreted into each medium by both species. Finally, growth of *S. costatum* and *H. akashiwo* in the bi-algal cultures was simulated using a mathematical model. This interaction model indicated that, as time passes, *S. costatum* and *H. akashiwo* steadily approach a stable equilibrium point of about 3.4 10^5 cells/ml and 4.8 10^5 cells/ml, respectively, when the two species coexist.

PO.09-09
Laboratory and field studies on harmful effects of large-scale HABs in the East China Sea

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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To understand the harmful effects of large-scale HABs in the East China Sea, a series of experiments and field investigations have been carried out since 2002. Laboratory experiments were conducted on the effect of HAB causative species, *Prorocentrum donghaiense* and *Alexandrium catenella* on the survival, feeding and reproduction of different organisms: juvenile perch, mysids, copepods, eggs and larvae of scallop, rotifer, *Moina mongolica* and *Artemia salina*. It was found that *A. catenella* at the bloom density of 10^5 cells/mL had an adverse impact on all above organisms. *Prorocentrum donghaiense* at lower density could be utilized as food by small zooplankton species, but inhibit their reproduction at the bloom density of 10^7 cells/mL. *In situ* experiments showed that survival of the copepod *Calanus sinicus* was inhibited significantly by HAB seawater. *Prorocentrum donghaiense* influenced the protozoa community when added at the bloom density; PSP was detected giving a toxicity of 4.3 MU/L during *Alexandrium* bloom. However, few toxic shellfish samples were detected near the HAB area islands; several simulating food chains have been set up and the results showed that PSP from *Alexandrium* species could be transferred to fish and lobster.

PO.06-27
Were nutrients a driving cause for the formation and disappearance of a *Scrippsiella trochoidea* red tide in a marine bay of Hong Kong

Session: PO.06 – Population dynamics

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A semi-enclosed bay in Hong Kong is one of hot spots for red tides (high biomass blooms). However, concentrations of ambient nutrients are not high enough to support such high biomass. When a red tide of *Scrippsiella trochoidea*

occurred in the bay, we investigated the field conditions of the red tide and conducted nutrient addition experiments on water samples from the red tide and non-red-tide waters to examine whether the red tide would be sustained with and without added nutrients or would collapse rapidly in bottles. Chl *a* was high in the red tide patch, but nutrients (NO_3, PO_4, SiO_4, and NH_4) were all low in the non-red-tide waters, suggesting that nutrients are not a direct driving cause for the formation of this high biomass red tide. During the incubation, phytoplankton biomass gradually decreased over 9 days in the red tide water samples without nutrient additions and without N additions and steadily increased with N additions until day 7. In the non-red-tide samples, additions of all NO_3, PO_4, and SiO_4 did not produce high *S. trochoidea* biomass, indicating that factors other than nutrients promoted the red tide formation of this species.

PO.14-14
Control of cell growth of cyanobacterial cells using extract from water grasses and leaves of evergreen trees

Session: PO.14 - Mitigation

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Growth inhibitors against cyanobacteria were identified in extractions from various plants,and applied to small surfaces of water such as drinking water reservoirs and recreational lakes. It is well known that cyanobacterial blooms do not form in some eutrophic ponds. In these cases, leaves of trees around the ponds fall into the ponds, or some macrophytes grow in the ponds. As a possibility, we thought that water-extractable compounds from the macrophytes and /or the leaves affect the cell growth of cyanobacteria.
In order to examine the cell growth inhibitory activity of water extractable compounds from the macrophytes and the leaves, various plants were collected and extracted with water. We found several inhibitory active compounds from wood, flower petals and young leaves. The compounds were isolated and purified from the extracts for identification of chemical structure

PO.16-04
Overwintering of *Heterocapsa circularisquama* (Dinophyceae) as a pellicle cyst induced by low temperature in the laboratory

Session: PO.16 – Life cycles

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Red tide blooms of *Heterocapsa circularisquama* Horiguchi cause mass mortality of bivalves and present a serious problem for shellfish aquaculture in western Japan. Sexual reproduction has not been found in *H. circularisquama*, and the mechanism of overwintering of the populations blooming in Japanese waters is unclear. Dinoflagellate temporary cysts are non-motile and formed from vegetative cells by shedding of the theca (pellicle cyst). We propose that the pellicle cyst of *H.*

circularisquama is a temporary stage rather than a resting stage. *Heterocapsa circularisquama* grew well at ca.17.5 °C, poorly at 15 °C and 12.5 °C, and it died at 10 °C. Two weeks after inoculation, the ratio of pellicle cyst/total cells at 12.5 °C was 57.5±1.4 %, significantly higher than at the other temperatures. After 60 days and 90 days of incubation at 10 °C under continuous darkness, viability percentage of the pellicle cyst was 0.9% and 0.8 %, respectively. Thus, the pellicle cyst designated as temporally cyst in the other dinoflagellates is not always in a temporary stage in *H. circularisquama*, part of a *H. circularisquama* population might overwinter as pellicle cysts formed at low temperature.

PO.13-25
Long-term variation of phytoplankton in Harimanada, Seto Inland Sea, Japan

Session: PO.13 - Regional events

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Chattonella antiqua was responsible, in the 1970-1980s, for large fishery damages in Harimanada, Seto Inland Sea, Japan. PSP toxicity is also a problem in this region. This research was carried out to clarify the appearance of phytoplankton, including harmful and toxic species. Long-term variations in phytoplankton a point in Harimanada were investigated from Apr. 1983 to Dec. 2005, three to five times a month during summer, and one to three times a month at other seasons. A litre of seawater was collected from 8 layers at 5-m intervals, filtered and concentrated to 50 mL. The number of phytoplankton species in the unfixed samples was counted. Among the species observed in this study, harmful and toxic species found were: *Chattonella antiqua*, *C. marina*, *C. ovara*, *C. verruculosa*, *Heterosigma akashiwo*, *Karenia mikimotoi*, *Cochlodinium polykrikoides*, *Heterocapsa circularisquama*, *Alexandrium catenella*, *A. tamarense*, *A.tamiyavanichii*, *Gymnodinium catenatum* and *Dinophysis fortii*.

PO.05-20
Preparation of toxin standards for use in monitoring diarrhetic shellfish toxins by LC-MS

Session: PO.05 - Toxin analysis

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In determining marine toxins in seafood, LC-MS is the most powerful tool with its high sensitivity, specificity, and accuracy. To promote wide use of the method, however, an adequate supply of standard toxins is imperative. Though highly or moderately contaminated shellfish have been unavailable for years in Japan, we succeeded in preparing DSP-toxin standards by taking different approaches. First, we obtained okadaic acid (OA) and dinophysistoxin-1 (DTX1) by culturing *Prorocentrum lima*. Second, OA and DTX1 were extracted from the black sponge, *Halichondria okadai*. Third, in order to secure DTX1, which was of low occurrence in the preceding two sources, we improved purification procedures to enable purification of DTX1 from scallop extracts of very low DTX3 contents. Fourth, we improved reaction conditions to synthesize from OA and DTX1 corresponding 7-*O*-palmitoyl esters. The structure and purity of the standard toxins thus prepared were confirmed by HPLC-DAD, 1D- and 2D-NMR, and ESI-MS. The toxin quantity in a parent stock was determined by weight.

PO.14-02

A successful control of HABs by modified clay: mitigation of Cyanophyta blooms in Xuanwu Lake in Nanjing

Session: PO.14 - Mitigation

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A bloom of *Microcystis aeruginosa* occurred in Xuanwu Lake in June, 2005, which had serious impact on landscape, water quality and the living conditions of residents. It also posed as a threat to aquatic sport activities of '10th national games', scheduled to take place in the lake. Based on the theory of clay surface modification, clays with high coagulation efficiency were made and disseminated in different ratios, frequency and concentration, matching the distribution characteristics of the blooms in different lake areas. The blooms were controlled effectively. Monitoring showed that the average removal rate of *Microcystis aeruginosa* was more than 96% and the coagulated cells were decomposed gradually. The quality of lake water had significantly improved. The community structure of phytoplankton had changed. There was no visible negative impact on the lake, such as dead fish, shrimp or water plants.
This represents a successful example of mitigation of lake blooms. It improved the water quality for the benefit of the residents and ensured that the aquatic sport activities of the '10th national games' could proceed normally as scheduled.

PO.05-33

Analysis of toxins responsible for poisoning incidents caused by consumption of the snail *Nassarius* spp.

Session: PO.05 - Toxin analysis

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During 2002 and 2003, poisoning incidents caused by the consumption of snail *Nassarius* spp. were reported in Fujian Province and Jiangsu Province, China. After observation of the symptoms in the victims it was suspected that PSP toxins were involved, and the toxicity screening of the snail samples with mouse bioassay method for monitoring of PSP toxins gave numbers exceeding 10,000MU/100g tissue (wet weight). However, no PSP toxins were detected after analysis of snail samples using high performance liquid chromatography (HPLC) with post-column derivatization. The toxins in *Nassarius* spp. were finally identified as tetrodotoxin (TTX), after re-analysis of the samples using HPLC coupled with a mass detector. The derivatives of TTX in toxic snail samples were also analyzed, using HPLC coupled with a triple quadrupole mass detector (API 4000), in the mode of precursor ion scan and product ion scan. Two isomers of trideoxyTTX, two isomers of TTX, two isomers of 11-oxoTTX, anhydroTTX and trace amounts of deoxyTTX were identified from the toxic snail samples. Analytical methods using select ion monitoring and select reaction monitoring targeted on these compounds were then developed.

PO.11-11
Roles of macroalgae for HAB mitigation

Session: PO.11 - Allelopathy

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The effects of two macroalgal species, *Ulva pertusa* and *Gracilaria lemaneiformis*, on the growth of microalgae in co-cultured systems were studied. Both species, especially their fresh tissues, significantly impeded the growth of *Heterosigma akashiwo*, *P. donghaiense* and *A. tamarense*. *Ulva pertusa* affected the growth of microalgae more than did *G. lemaneiformis*. A positive correlation between the initial macroalgal biomass and their effects on microalgae during co-culturing was observed. Nutrient assays showed that when microalgal cells were dead, nitrate and phosphate were almost exhausted in the *G. lemaneiformis* co-culture, but remained at enough levels in the *U. pertusa* co-culture to allow for further growth of the microalgae. Therefore, allelopathy was the essential factor for the inhibition of *U. pertusa* on the microalgae, while the combined roles of allelopathy and allelospoly may be responsible for that of *G. lemaneiformis*. In a co-cultured system of *G. lemaneiformis* with *Scrippsiella trochoidea* the simultaneous nutrients assay also revealed that allelospoly might be the main reason for inhibition of *S. trochoidea* under the lower initial ratio of *G. lemaneiformis* to *S. trochoidea.* However, both allelopathy and allelospoly could be responsible for the inhibition of *S. trochoidea* at higher initial biomass ratio.

PO.09-03
Sulfotransferase activity in PSP-producing *Alexandrium* species

Session: PO.09 - Toxin synthesis and chemical structure of toxins

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Sulfotransferase (ST) is an important modified enzyme related to toxin conversion in PSP-producing dinoflagellates. This study investigated ST activity in the crude extracts of eight *Alexandrium* strains with various toxin compositions. The toxin biosynthesis pathway in *Alexandrium* was also examined. ST activity was only detected in the crude extract of *Alexandrium tamarense* CI01 (ATCI01), which was able to convert GTX2/3 to C1/C2, while it was not found in other PST-producing *Alexandrium* strains, even the strains with similar toxin composition as ATCI01. Moreover, ST activity was not detected in the non-toxic *A. tamarense* CCMP2023. These results indicated that ST is a species/strain-specific enzyme and that different toxin biosynthesis pathways may exist in different PST-producing *Alexandrium* strains. This requires further study at the molecular and biochemical level.

PO.15-03
Relationship of magnitude and position of the algal SICF with chlorophyll-a concentration

Session: PO.15 - Monitoring

Dong-Zhi Zhao

National Marine Enviroment Monitoring C, DALIAN, China

To investigate the relation of sun-induced chlorophyll fluorescence near 685nm with chlorophyll-a concentration, the reflectance spectra of red tide species such as *Gymnodinium sp*., *Heterosigma akashiwo*, *Ceratium furca* and other algae such as *Nitzschia closterium*, *Dicrateria zhanjiangensis* Hu.,*Platymonas sp*., *Chlorococcum sp*., *Platymonas helgolandica* var. *tsingtaoensis*, *Chlorella sp*. from field samples and laboratory cultures was used. R_{max}red normalized at R560 correlated well with chlorophyll-a concentration (R>0.82). To different algae, the coefficient a of the relationship between R_{max}red/R560 and chlorophyll-a presented change. The regression equation (Rmaxred/R560 =a (chl a) b) reveal that the coefficient a varied between 0.037 and 1.135, b varied between 0.094 and 0.727. Simultaneously this study established the relationship between the fluorescence peak position and chlorophyll-a concentration. All regression coefficients were higher than 0.75 except *Chlorococcum sp*. with 0.57. The position of the fluorescence peak shifted to infrared when chlorophyll-a concentration increased, but for various species, the shift velocities of peak positions were different. Thus *Heterosigma akashiwo* was the fastes and in other algae the shift was between 0.1 and 0.3nm per 10mgm^{-3}. No shift was observed in *Dicrateria zhanjiangensis* Hu and *Chlorococcum sp*.

PO.11-12
The role of allelopathy in diatom and dinoflagellate blooms in the East China Sea

Session: PO.11 - Allelopathy

Weihong Zhao[1], Meimei Chen[1], Jiangtao Wang[2]

[1]Institute of Oceanology, CAS, QINGDAO, China
[2]Ocean University of China, QINGDAO, China

Skeletonema costatum, Karenia mikimotoi and *Prorocentrum donghaiense* blooms took place successively in the East China Sea in the spring of 2005. Bloom water was collected and filtered. *Skeletonema costatum*, *Pseudo-nitzschia pungens*, *Alexandrium tamarense*, *P. donghaiense*, and *Prorocentrum micans* were cultured in the above filtered solutions to explore the role of allelopathy in the phytoplankton community succession. The results showed that the water collected from both the *S. costatum* bloom and the *K. mikimotoi* bloom stimulated growth of the five tested species. Mixed water from *K. mikimotoi* and *P. donghaiense* blooms stimulated growth of all tested species except *S. costatum*. The stimulatory effect of *K. mikimotoi* on *P. donghaiense* was most marked and intensified with the concentration of *K. mikimotoi*. Thus the stimulus produced by *S. costatum* on *K. mikimotoi* and *P. donghaiense* may give it an advantage in later *K.*

mikimotoi and *P. donghaiense* blooms. In the same way, some stimulatory substances excreted by *K. mikimotoi* on *P. donghaiense* may play an important role in the succession from *K. mikimotoi* bloom to *P. donghaiense* bloom.

PO.13-13
The low temperature characteristics of the East China Sea in early spring of 2005 and its influence on HABs

Session: PO.13 - Regional events

Dedi Zhu[1], Xianwei Bu[1], Yunfeng Wang[2], Weiyi Xu[1], Jilan Su[1]

[1]Second Institute of Oceanography, SOA, HANGZHOU, China
[2]Institute of Oceanography, CAS, QINGDAO, China

Temperature characteristics of a HAB-frequent area in the East China Sea in early spring 2005 and its influence on a large-scale HAB are analyzed, based on survey data from 2002~2005 and some related historical meteorological data. Low temperature characteristics were obvious in the HAB- frequent area during the early Spring (early April) 2005; water temperature was about 2-3 °C lower compared to the same period of 2004 and it quickly rose from 10 °C to 17 °C during the last twenty days of April. Low air temperature in March and large discharge of the Yangtze river in Winter (Jan. to Mar.) in 2005 are the main causes for the low temperature in this sea area. The low temperature may have been an important factor for the occurrence of a dinoflagellate HAB in the spring 2005 as opposed to the spring 2002~2004.

PO.07-12
Numerical simulation of circulation and its application in red tides in the Changjiang River Estuary and adjacent sea areas

Session: PO.07 - Ecology and oceanography

LS Zhu, XH Chen

South China Sea Institute of Oceanology, GUANGZOU, China

Based on the COHERENS, a three-dimensional baroclinic model for the summer of East China Sea was established with the sigma-coordinate in the vertical direction and spherical coordinate in the horizontal direction. The circulation patterns of the Kuroshio Current, the Taiwan Warm Current, the Tsushima Current, the Changjiang Diluted Water and the coastal currents were successfully simulated in this model. The calculated results are fairly consistent with previous observations and studies. Based on this baroclinic current field, we simulated the Lagranian particles tracking to estimate possible origins of the regions where red tides frequently occurred in the Changjiang River Estuary and adjacent sea areas. Does seeding from cysts take place from red tide algae in the seabed of the Taiwan Strait, the offshore area of Fujian and Zhejiang Provinces and the northeast Taiwan Island, to the Changjiang River Estuary and adjacent sea areas. Field data are needed to confirm this. The building of a numerical model based on the COHERENS to simulate the Lagrangian particle tracking for estimating the source of the red tides is the first attempt of this kind in China.

PO.01-16
Diversity in the genus *Skeletonema*: an overview

Session: PO.01 - Genetics

A Zingone, D Sarno, WHCF Kooistra

Stazione Zoologica A. Dohrn, NAPLES, Italy

Recently, the genus *Skeletonema* has undergone profound revision, which has revealed four new species within one of the most conspicuous marine phytoplankton taxa, *S. costatum*. Morphological and molecular analyses have now been extended to more than 150 strains from all over the world with the aim of a) assessing the diversity of the genus, b) investigating the morphological and molecular variability of the different species, and c) depicting their geographic ranges. This study has uncovered live material of *S. costatum* and *S. grevillei*, which were previously only known from their type material collected in Hong Kong waters 130 year ago. In addition, a new species has been found in material from the Pacific Ocean. The analysis of strains from different areas has revealed consistent genetic diversity (LSU data) within some species (e.g. *S. dohrnii*, *S. menzelii*, *S. tropicum*), which still retain monophyly and separation from closely related species. Other species (*S. marinoi* and *S. japonicum*) are instead homogeneous over their range. Despite the limited number of strains available for each species, geographic patterns are recognisable, which differ among species and among genetically distinct populations within morphologically defined species. The possible origin and ecological significance of cryptic diversity in *Skeletonema* is discussed.

SOCIAL PROGRAMME

• Monday 4 September
Reception hosted by the City of Copenhagen.

Participants must be at the main entrance to the Town Hall at 06.00 pm. It is a 10 min walk from the Conference Centre.

• Thursday 7 September
ISSHA General Assembly and Auction at DGI – byen.

See elsewhere in Programme.

• Friday 8 September
Mermaid Dinner Party, Langelinie Pavillionen

The congress banquet will take place at Langelinie Pavillionen situated at Langelinie, very close to the Little Mermaid.

Transportation will be by canal boats, which will take you on a picturesque and guided tour through the canals of Copenhagen. The duration of the trip is about 30 min.

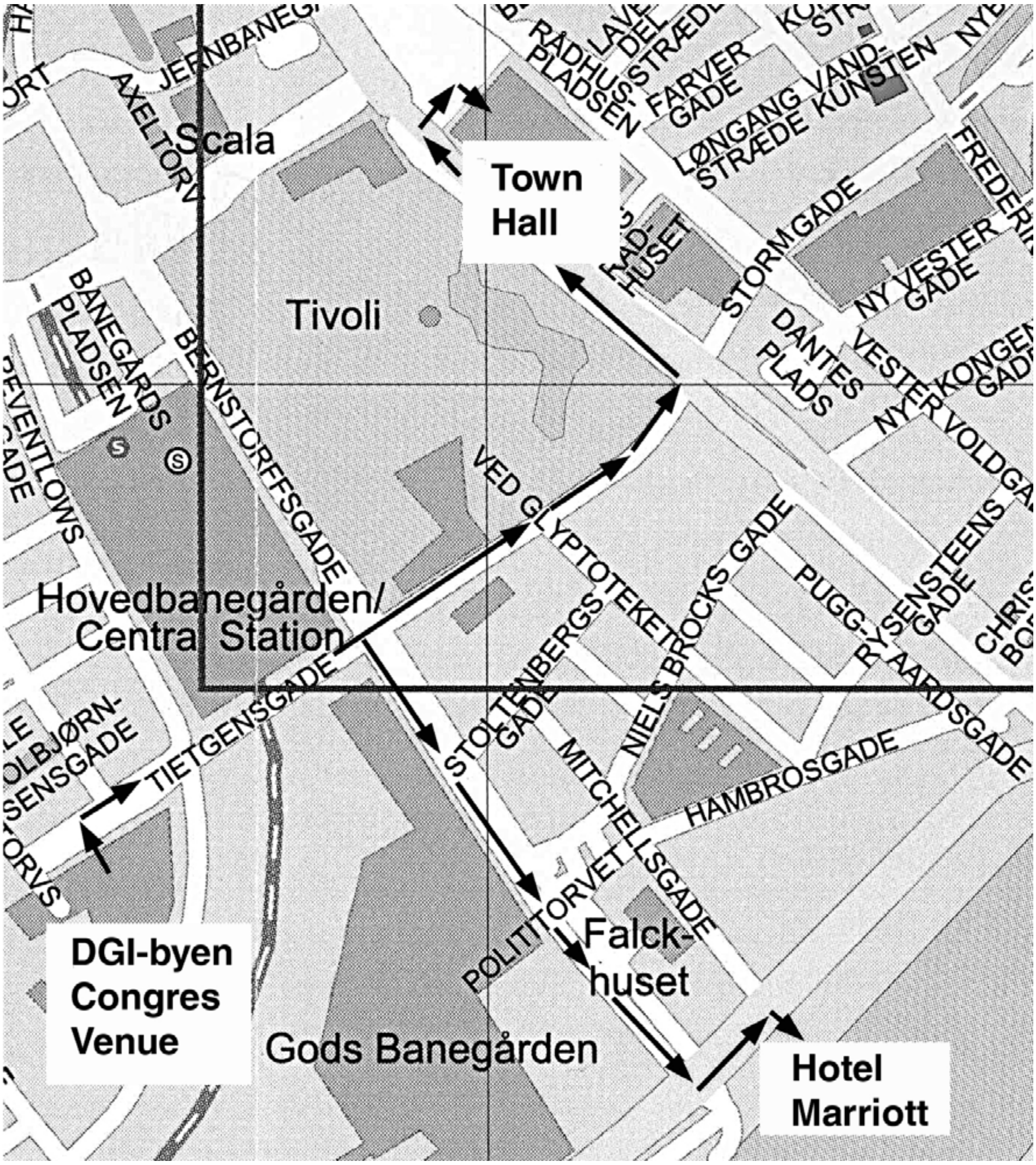
The boats will leave from Hotel Marriott situated at Kalvebod Brygge (close to the Congress Venue see map) at 7:00 PM.

Should you miss the boat or prefer to get to Langelinie Pavillionen on your own:

1. Taxi: costs ca. 150 DKr.
2. Bus: line A1 from Tietgensgade to Esplanaden and about 5-10 min walking from the bus stop to Langelinie.
3. Walk: about 40 min. from the Congress Venue

The Dinner begins at 7:45 PM. There will be busses going back to the city centre (not each hotel, but close) at 11.00 pm and at midnight. For those who wish to stay longer and dance and talk there is a 15 min walk back or you can order a taxi. The party closes at 02.00 am.

For map see next page:


Scala
Town Hall
Tivoli
Hovedbanegården/ Central Station
DGI-byen Congres Venue
Gods Banegården
Falck-huset
Hotel Marriott
JERNBANEGADE
AXELTORV
RÅDHUSPLADSEN
FARVERGADE
LØNGANGSTRÆDE
STORMGADE
NY VESTERGADE
DANTES PLADS
VESTER VOLDGADE
NY KONGENSGADE
BANEGÅRDSPLADSEN
BERNSTORFFSGADE
REVENTLOWSGADE
VED GLYPTOTEKET
STOLTENBERGSGADE
NIELS BROCKS GADE
PUGGÅRDSGADE
RYSENSTEENSGADE
TIETGENSGADE
MITCHELLSGADE
HAMBROSGADE
POLITITORVET
AARDSGADE

Excursions (pre-booked)

Social tours on Wednesday, September 6

Tours start immediately after closing of the symposia.

The City, the Canals and the Opera House (4 h. incl. lunch bag)
Copenhagen was - for Hans Christian Andersen - “Wonderful, wonderful Copenhagen, friendly old queen of the sea” and this tour is one of the best ways to see what he meant. During this charming tour of the city, you will see many of the main points of interest within the city: City Hall Square, the Carlsberg Glyptotek and Tivoli Gardens, the National Museum and the old “Latin Quarter” - so called because here you find the university and academic centre of old Copenhagen, - the Round Tower and the Old Fish Market. Here, you board our chartered, specially built canal launches and cruise through the canals and harbour. You will see Copenhagen as sailors have seen it for several centuries, passing under the incredibly low bridges to view some of the fabulous buildings that - typically for a city with maritime associations - are all close to the sea or the waterways: Christian IV’s Stock Exchange, Christiansborg Palace and Holmens Church - the Naval church in Copenhagen - and the charming old sailor’s quarter Nyhavn, Amalienborg Palace and the Little Mermaid. The Canal tour ends at the new magnificent Opera House that has been donated to the people of Denmark by the world largest shipping company Maersk.

Kings and Vikings - a step back in history (4 h. incl. lunch bag)
The ingredients of this tour are the tough, redbearded warriors and traders who marauder, conquered and traded with most of maritime western Europe and their descendants, Royal or commoner. The tour takes you to Roskilde, where you will visit the Viking Ship Museum which houses the remains of 5 Viking ships salvaged from the bed of the fjord and painstakingly preserved. A short drive to the centre of Roskilde, for a visit to the twin-spied Cathedral: until the early 15th century, Roskilde was the capital of Denmark - proof of its strategic importance in the early Middle Ages - and all Danish monarchs were buried here. Although no longer the capital, Danish Kings and Queens are still laid to rest in Roskilde.

The Castles of North Sealand (4 h. incl. lunch bag)
This marvellous outing takes you to North Sealand - the beautiful countryside north of Copenhagen to Fredensborg to see Fredensborg Palace which is the Spring and Autumn residence of the Queen of Denmark and the residence of the Crown Prince. The tour continues to the small town of Hillerød where you will be confronted with one of the dominant figures amongst Danish monarchs - Christian IV and his Frederiksborg Palace. Built on a small island in a lake, Frederiksborg Palace is a magnificent Renaissance palace - beautifully situated and unbelievably detailed, which today houses the Museum of Danish National History.

Rules & Conditions for Social tours

All tours are guided in English. During transportation participants are insured according to Danish Legislation regarding Transportation Insurance, but should otherwise be covered by their personal travel and health insurance when not on the bus.
All tours will start/end in front of DGI-Byen (the Venue) at the time mentioned or on the ticket. You are kindly asked to announce your presence at least 10 minutes prior to the departure time. To board the bus you have to present a valid ticket, otherwise the tour guide is authorised to refuse admittance on the tour. Cancellation of any tours must be made in writing and forwarded to DIS Congress Service at least 21 days prior to operation of tour. After this, no refund can be expected. A cancellation fee at 10% of the price applies for any cancellation.
The Congress Bureau reserves the right to adjust or change the programme as necessary. A minimum advance reservation for 20 persons per tour is required in order to guarantee operation. The Congress Bureau reserves the right to cancel operation in the event of insufficient advance reservations. In the event that the Congress Bureau cancels the tour full reimbursement will be made.

ISSHA GENERAL ASSEMBLY AND AUCTION

Proposed Agenda for the General Meeting of The Society for the Study of Harmful Algae held at the 12th International Conference on Harmful Algae

7 September 2006

Call to order — Pat Tester, President

Approval of minutes of last meeting — Tracy Villareal, Secretary

(Minutes will not be read but are available on the ISSHA website)

Treasurer's report — Nina Lundholm, Treasurer

Committee reports by Chairs

- Travel awards — Don Anderson
- Publications — Jane Lewis
- Finance — Nina Lundholm
  - (Topics not covered in Treasurer's Report)
- Achievement awards — Barrie Dale
  - (Review of rules - Awards will be announced at the Mermaid Banquet)
- Membership — Pat Tester
- Elections — Karen Steidinger
- Ad hoc — Henrik Enevoldsen/Tester/Zingone
- Conference program — Øjvind Moestrup

Special Orders

- Venue for 2010 meeting, presentations from candidates and ballot
- Presentation of 2008 venue, Hong Kong, China.

Unfinished Business

New Business

Announcements

Adjournment

Conference ISSHA Committee Chairs and Members

Committee on Statutes and Bylaws
It was decided not to change the Statutes and Bylaws for now, but to do so once there was a greater need. Therefore, no Chair was appointed. For now, the Council will remain responsible for this activity and will recruit other members when enough proposed changes accumulate, to justify revising the Statutes and Bylaws.

Committee on Elections
Chair: Karen Steidinger
Member: Stephen Bates

Committee on Membership
Chair: Pat Tester
Members: Kim Hak-Gyoon, Beatriz Reguera, Gustaaf Hallegraeff and Ted Smayda

Committee on Finances
Chair: the current Treasurer, Nina Lundholm
Members: Don Anderson, Karen Steidinger, Henrik Enevoldsen, Yasuwo Fukuyo, Edna Granéli

Committee on Conference Program
Chair: Øjvind Moestrup
Members: Don Anderson, Allan Cembella, Barrie Dale, Greg Doucette, Henrik Enevoldsen, Gustaaf Hallegraeff, KC Ho, Jane Lewis, Pat Tester, Adriana Zingone, Minjiang Zhou

Committee on Achievement Awards
Chair: Barrie Dale
Members: Allan Cembella, Henrik Enevoldsen, Beatriz Reguera, Pat Tester and Marina Montresor

Committee on Travel Awards
Chair: Don Anderson
Members: Allan Cembella, Henrik Enevoldsen, Beatriz Reguera, Karen Steidinger, Yasuwo Fukuyo and Edna Granéli

Committee on Publications
Chair: Jane Lewis
Members: Stephen Bates, Alan Cembella, Barrie Dale, Greg Doucette, Henrik Enevoldsen, Karen Steidinger, Pat Tester, Adriana Zingone

Ad hoc Committee on Special Projects
Co-chairs: Henrik Enevoldsen, Pat Tester, Adriana Zingone

Executive

President: Pat Tester
pat.tester@noaa.gov

Vice President:
Gustaaf Hallegraeff
hallegraeff@utas.edu.au

Vice President:
Beatriz Reguera
beatriz.reguera@vi.ieo.es

Secretary:
Tracy Villareal
tracy@utmsi.utexas.edu

Treasurer:
Nina Lundholm
nlundholm@bi.ku.dk

Past President:
Karen Steidinger
Karen.Steidinger@MyFWC.com

Council (2004-2007)

Donald Anderson (USA)
Barrie Dale (Norway)
Greg Doucette (USA)
Yasuwo Fukuyo (Japan)
Edna Granéli (Sweden)
Hai-Gyoon Kim (S. Korea)
Jane Lewis (UK)
Øjvind Moestrup (Denmark)
Marina Montresor (Italy)
Ted Smayda (USA)
Adriana Zingone (Italy)

Statutes

These Statutes apply only to the global organization of the International Society for the Study of Harmful Algae.

Article 1. The name of the Society shall be "The International Society for the Study of Harmful Algae" (ISSHA), hereafter referred to as "the Society".

Article 2. The Society shall be an international non-governmental, non-political and not-for-profit organization.

Article 3. The Society shall be affiliated to the International Council for Science (ICSU) family of organizations as a constituent part of the International Union of Biological Sciences (IUBS), through the International Association of Biological Oceanography (IABO). The Society shall have observer status with the Intergovernmental Oceanographic Commission (IOC) of UNESCO.

Aims and Objectives

Article 4. The aims of the Society shall be to advance understanding by the promotion and pursuit of all aspects of the study of harmful algae and to widely disseminate the results of this research.

Article 5. The objectives of the Society shall be to:

i. promote the study of harmful algae, including their occurrence, related oceanographic factors, ecophysiology, taxonomy and systematics, genetics, toxin chemistry, toxicology, and management and mitigation;
ii. collect, evaluate and disseminate information on harmful algae;
iii. promote harmful algal research, projects, programs and training, and extend these activities to foster the related subjects of harmful algal bloom management and mitigation;
iv. promote public awareness of the social, economic and ecological effects caused by harmful algae;
v. arrange and co-sponsor national, regional and international conferences, seminars, symposia and working group meetings;
vi. co-sponsor lectures and courses, and the publication of scientific and popular articles, books and proceedings;
vii. encourage the participation and training of students in the study of harmful algae;
viii. support and implement research projects and programs linked to the study of harmful algae.

Membership

Article 6. The membership of the Society shall be open to anyone interested in promoting the aims and objectives of the Society.

Article 7. Membership of the Society shall comprise the following categories:

i. **Honorary Members**: such members will be elected by the Council, on the recommendation of the Executive Committee, in recognition of their outstanding services to the furthering of the objectives of the Society;
ii. **Institutional Members**: such members will be from government organizations, academic and research institutions, professional societies, public and private industries, and bilateral and international organizations;
iii. **Individual Members**: such members will be from any individuals interested in promoting the aims and objectives of the Society.

Article 8. All applications for membership, with the appropriate registration fees, should be made directly to the Treasurer of the Society. The Treasurer will acknowledge the application and supply the applicant with a copy of the Statutes and pass the applicant's registration details to the Secretary. The payment of the annual subscription shall entitle the member to all privileges of the Society for that calendar year.

Article 9. Fully paid-up members shall have the following privileges:

i. receive all notices pertaining to the activities of the Society and to attend all Society-sponsored meetings, workshops and excursions at an appropriate reduced charge;
ii. receive all literature issued by the Society, free or at a reduced rate, as the Council may from time to time determine;
iii. vote in the election of the members of the Council, and on the conduct of the affairs of the Society at any meetings of the Society;
iv. be eligible for service on the Council and its committees, or as Officers;
v. introduce visitors at any meeting of the Society (with the exception of the General Assembly) unless the Council, by resolution, suspends this privilege for any particular reason.

Article 10. Resignation of membership shall be signified in writing to the Treasurer or by failure to pay the membership dues for two successive years.

Annual Subscription

Article 11. Annual membership subscriptions shall be payable in advance and shall be due on 31st December each year. Subscription rates shall be publicized in all relevant publications of the Society and on the ISSHA website.

Article 12. Honorary members are not liable to pay membership subscriptions.

Article 13. At the discretion of the Council, reduced subscription may be paid by the following categories of members:

i. student members in full-time education (as certified by their supervisor);
ii. members retired from full-time employment.

Article 14. If any member shall be in arrears of his/her subscription for one year, the Treasurer will advise the member of the fact and, if payment is not made before the end of the subsequent period of one year, the member's name will be removed from the list of members.

Governance

Article 15. The governance of the Society shall be by the following bodies:

i. the General Assembly;
ii. the Council;
iii. the Executive Committee.

Article 16. The **General Assembly**:

i. shall be comprised of all members of the Society, 10% of members to constitute a quorum;
ii. shall meet at least once every three years;
iii. shall be chaired by the President.

Article 17.

i. Notice of the General Assembly shall be sent to each member by the Secretary at the earliest possible date (normally at least one year in advance), and the agenda of the meeting shall be sent out at least one month before the meeting, and posted on the ISSHA website.
ii. At the General Assembly, members of the Society present shall consider any business brought before them by the Council, or by any member (of which notice in writing has been given to the Secretary at least two weeks before distribution of the agenda). The agenda will also include the election of the **President** of the Society, two **Vice-Presidents**, the **Secretary**, the **Treasurer** and **Members of Council** and approval of the triennial program and budget.
iii. At the General Assembly, all members (with the exception of the President) shall have one vote each. In the case of equality of votes, the Chair may exercise a casting vote.

Article 18. The **Council**:

i. shall be comprised of the President, Vice-Presidents, the Secretary, the Treasurer, the immediate Past-President (*ex-officio*) and a minimum of 4 and a maximum of 14 Members, 60% to constitute a quorum;
ii. shall meet at intervals not greater than two years, and yearly if practicable;
iii. shall be chaired by the President.

Article 19.

i. The general business of the Society shall be conducted by the Council.
ii. At the Council meetings, each Council member (with the exception of the Chair) shall have one vote. In the case of equality of votes, the Chair may exercise a casting vote.
iii. All members of the Council, except the immediate Past-President, shall be elected by written ballot of the members, the result of the ballot being declared at the General Assembly. The Council shall have the power to appoint any member of the Society to fill vacancies arising between elections; the tenure of such co-opted members shall terminate at the next election. No one may be appointed as a co-opted member if, as a result, more than one third of the members of Council would be co-opted members.
iv. The Council may set up standing or *ad hoc* committees for specific purposes, consisting either wholly or in part of Council members, provided that all acts and proceedings of any such committees shall be fully and promptly reported to the Council.

v. At the request of any three members, the Secretary shall convene a meeting of the Council, stating the nature of the business to be discussed.
vi. Meetings of the Council may be in person or by electronic media.

Article 20. The **Executive Committee**:

i. shall include the President, the two Vice-Presidents, the Secretary and the Treasurer; 60% to constitute a quorum.
ii. shall be chaired by the President.

Article 21.

i. The Executive Committee shall be responsible for decisions involved in implementing the Society's policies, between meetings of the Council.
ii. At Executive Committee meetings, each member of the Executive Committee will have one vote, a majority vote deciding the issue, and ties referred to the Council for resolution.
iii. Meetings of the Executive Committee may be in person or by electronic media.

Article 22. The **Officers of the Society** shall be elected for three years and will be eligible for re-election for one further term, after which they shall be ineligible for the same office for a period of three years. The Officers of the Society shall comprise:

i. The **President**, who shall chair the General Assembly, the Council and the Executive Committee. The immediate Past-President will continue to serve on Council (*ex-officio*) until succeeded by the next retiring President.
ii. The **Vice-Presidents**: in the absence of the President, one of the Vice-Presidents, chosen by the Executive Committee, will chair the meetings of the General Assembly, the Council and the Executive Committee.
iii. The **Secretary**, who shall be responsible for maintaining communications among the Executive Committee, the Council and the General Assembly. This task will also include the dissemination of information to the general scientific community, other non-governmental agencies and to the public, involving both written material and electronic communication (e.g. ISSHA web pages, electronic mailing list). The Secretary will maintain the list of Society members and keep the Society membership records.
iv. The **Treasurer**, who shall be responsible for the financial matters of the Society. The Treasurer shall provide an annual, audited account.
v. The **Members of Council**, who may take on specific duties as directed by the Council.
vi. A member will not normally hold more than one office at one time.

Elections and Ballots

Article 23. Elections

i. Any member of the Society may nominate candidates, who shall be members of the Society, for the election as President, Vice Presidents, Secretary, Treasurer or Members of Council.
ii. All such nominations, with the name of a seconder and with written consent of the nominee to act if elected, shall be forwarded to the Secretary no later than four months before the General Assembly. If no nomination is received for an

office falling vacant, it shall be the duty of Council to make such appointments.

iii. Voting shall be by written ballot. For this purpose, the Secretary shall circulate ballot forms to all members of the Society three months before the General Assembly. At that meeting, the ballot forms shall be opened and the count made by scrutineers appointed by Council, and the results of the ballot shall be declared.

iv. Where nominees receive the same number of votes, the matter will be resolved by a further vote of the members present at the General Assembly.

Article 24. Ballots

i. When a written vote is necessary, Individual, Honorary and Institutional Members shall each have one vote.

ii. Matters of basic organization of the Society, including the election of the Council and of the Executive Committee, shall be determined by written ballot of all members.

Finances

Article 25. The revenue of the Society shall be derived from annual subscriptions paid by Members, the sale of published materials underwritten by the Society and financial donations and any other forms of assistance received to further its objectives.

Article 26. Membership contributions shall be determined by the Council and ratified by the General Assembly of the Society or by written ballot.

Article 27. The Society shall be registered in Copenhagen, Denmark.

Article 28. Annual Accounts

i. It shall be the duty of the Treasurer to prepare annual accounts as prescribed by current legislation.

ii. The annual accounts will be examined by an Independent Examiner, appointed by Council, who shall be an independent person who is believed by Council to have the requisite ability and practical experience to carry out competent examination of the records.

iii. Copies of the annual accounts and the independent examiner's report shall be sent to members and presented to Council annually.

Article 29. Expenses

i. Members of the Executive Committee and of the Council shall receive no payment for services.

ii. Members required by the Executive Committee to attend official meetings of the Society, or of other organizations on behalf of the Society, may receive reimbursement for *bona fide* travelling expenses and a daily subsistence allowance.

Article 30. On an *ad hoc* basis, on behalf of the Society, the Executive Committee may appoint staff members as necessary to carry out the mandates and functions of the Society.

Minutes

Article 31. The Council shall cause Minutes to be duly entered in the books for the purpose of recording: all the appointments to the Council; the names of the members present at each business meeting (General Assembly, Council, Executive Committee) of the Society; and the proceedings of these meetings. An Executive Summary of actions of committees and officers of the Society, between General Assembly sessions, shall be prepared by the Executive Committee. This summary shall be approved by majority vote at the General Assembly and be posted on the ISSHA website.

Alterations to the Statutes

Article 32. Proposed amendments to the Statues may be initiated by the Council or by a majority of the members of the Society, and shall be approved by the General Assembly, provided that two thirds of all the ballots received (by regular mail, fax or e-mail, and cast at the General Assembly) support the amendment. Any proposed amendments will be circulated to members with the agenda for that meeting. Between General Assembly sessions, amendments to the Statutes may be approved by written ballot, providing that two thirds of the ballots mailed in support the amendment.

Dissolution of the Society

Article 33. The Society may be dissolved, after one year's notice in writing to all members, by a two-thirds majority of the vote of the General Assembly or by a majority vote by written ballot of members.

i. Such a proposal for dissolution shall be initiated by the Council.
ii. On the dissolution of the Society, its assets shall be assigned to the International Union for Biological Sciences (IUBS) through the International Association of Biological Oceanography (IABO).

ISSHA AUCTION CATALOGUE

The auction will be held immediately after the ISSHA Assembly. Sandwiches will be served and beverages can be purchased in a bar set up for the Auction.

The proceeds of the Auction will go to ISSHA Travel Awards. So far the following items have been donated:

Donor	Items
Haruyoshi Takayama	Wood carvings
Aquanet	Plankton-net
Jellett Rapid Testing Ltd.	Rapid Test for DSP+Rapid test for ASP
Anon.	Books & local specialities
Elizabeth Fensin	Handmade jewelry-- two algal necklaces (glass beads)
Rut Akselmann	Oil painting
Nikon Microscopy Division in Denmark c/o DFA A/S	Nikon digital camera D50
KC Denmark Res. Equipment	Utermöhl counting chambers 2 x 10 ml, 2 x 25 ml & watersampler
Hunters House (Denmark)	Kunan fishing rod
Biosense Laboratories AS	ASP ELISA-kit
Prof. G. R. Hasle	Signed reprints and books (e.g. Hasle G.R. 1965. Nitzschia and Fragilariopsis species studied in the light and electron microscopes. II. The group Pseudonitzschia.)
Pat Tester	T-shirts and books
Sheean Haley (WHOI)	algal postcards
Jinhui Wang	Embroidery from Hunan Province, China
Padmakumar, K.	Indian art + Aromatic cashew alcohol
Maria Faust & Pat Tester	Dinoflagellate photos
Karen Steidinger	Nikon N60 reflex camera
Edna Granéli	'Proceedings of the IVth Int. Conf. on Toxic Phytoplankton, Lund', 'Ecology of Harmful Algae' (Granéli & Turner, eds)
Gbemi Akin-Oriola	tie and dye men's shirts and jewelry
Dept. of Phycology, Univ. of Copenhagen	Br. Phycol. J., vol. 4-27
Jane Lewis	'The scallop' (eds. Ian Cox)
Olympus, Denmark	Olympus SP500 digital camera

An updated and complete list of items on aucion will be circulated prior to the auction.

Payment can be be made cask in DKR, Euro or USD and by major credit cards

PRACTICAL INFORMATION

Venue: www.dgi-byen.dk
DGI-Byen
Tietgensgade 65
DK- 1704 Copenhagen V
Phone: + 45 3329 8128
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DGI-byen is located right behind Copenhagen's Central Station and is a popular venue for cultural and corporate events and sport. 22,000 square metres in area DGI-byen offers a range of conference facilities, café, restaurant, party and banquet rooms, and three hotels, in addition to all its other facilities: The Swim Centre, The Spa, The Bowling Alley.

The Swim Centre: Open: Monday - Thursday 06.30 - 24.00, Friday 06.30 - 19.00, Saturday and Sunday 09.00 - 17.00. Come and enjoy the facilities, and for as long as you like. There is no time limit. Swim in the super-ellipse pool. Try out the climbing and diving pool, or relax in the warm spa pool.

The Spa: Open: Monday - Thursday 09.30 - 21.00, Friday 09.30 - 19.00, Saturday/Sunday 09.30 - 17.00. Spoil yourself and recharge your batteries at the DGI-byen Spa. Our spa is all about wellness, relaxation and revitalisation and treatments to imbue new energy. Take a steam bath, sauna and a dip in the cold tub, to give your body a good old scrub. Various treatments are on offer at The Spa including aromatherapy, mud packs, facials and body massage.

Bowling: Open: Monday 16.00 - 23.00, Tuesday- Thursday 14.00 - 23.00, Friday and Saturday 11.00 - 01.00, Sunday 10.00 - 20.00. Go bowling at one of ten fully automatic lanes, and slake your thirst or have a snack while you're at it at Café Strike. To book lanes visit the Bowling Centre, or phone +45 3339 8020 or email a booking request to bowling@dgi-byen.dk. Please arrive 15 minutes before the time scheduled.

Conference Secretariat / Bureau

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Banks
Normal banking hours are from 09:30 to 16:00 hrs Monday through Friday. On Thursday banking hours are extended to 18:00 hrs. Extended banking facilities are available at Copenhagen Central Railway Station 7 days/week between 07:00 and

21:00 hrs. There are automatic cash dispensers, usually located in connection with a bank branch, which accept a variety of international credit cards. The cards accepted are indicated on the dispenser.

Shops
The shops are open from 09:30/10:00 to 17:30/18:00 hrs Monday through Thursday and until 20:00 hrs on Friday. On Saturdays the shops are open until 17:00 hrs and most shops are closed on Sundays.

Tips
Tips are always included in the prices given in taxies and restaurants.

Electricity
Electricity is supplied at 220 volts A/C, 50 Hz cycle.

Emergency Services
Police - Ambulance - Fire Brigade: Dial 112

LIST OF RESTAURANTS

At http://www.aok.dk/section/english you will find a comprehensive guide to everything about Copenhagen.

DANISH

For those wishing to taste perhaps the most outstanding of Danish gourmet food, the best time is at lunch when the unique 'smørrebrød' is served.

A world-famous lunch restaurant is Ida Davidsen. Here the choice of 'smørrebrød', Danish open sandwiches, is so vast that the menu is two meters/6 feet long!

Another great restaurant for smørrebrød is Slotskælderen hos Gitte Kik across from the Parliament, here spotting Danish MPs is a favourite sport.

Ida Davidsen
Store Kongensgade 70
Tlf. 33 91 36 55

Restaurant Mønten
Møntergade 24
Tlf. 33 13 33 74

Kanal-Kaféen
Frederiksholms Kanal 18
Tlf. 33 11 57 70

Slotskælderen hos Gitte Kik
Fortunstræde 4
Tlf. 33 11 15 37

Café Toldboden
Amaliegade 41
Tlf. 33 12 94 67

Restaurant Domhuskælderen
Nytorv 5
Tlf. 33 14 84 55

Det Lille Apotek
Store Kannikestræde 15, København K
Phone: 33 12 56 06

Told & Snaps
Toldbodgade 2, København K
Phone: 33 93 83 85

Cafe Sorgenfri
Brolæggerstræde 8, København K
Phone: 33 11 58 80

Cafe Charlottenborg
Nyhavn 2, København K
Phone: 33 13 11 58

Cafe & Ølhalle 1892
Rømersgade 22, kld., København k
Phone: 33 33 00 47

Den Danske Kro
Nørre Farimagsgade 13, København K
Phone: 33 11 15 13

Færgekroen
Vesterbrogade 3, Tivoli, København V
Phone: 33 75 06 80

Galionen
Nyhavn 23, København K
Phone: 33 32 09 99

Grøften
Vesterbrogade 3, Tivoli, København V
Phone: 33 75 06 75

BUDGET (see also under vegetarian)

Looking for a nice meal without overextending your budget?
Copenhagen offers many possibilities.

Christiania

The hippie town just across the bridges on the island of Amager is worthwhile visiting. The atmosphere is very laid back and informal. The cafés and restaurants go for organic food and the service is very relaxed.
French atmosphere

Are you looking for a quick meal downtown or a reasonable bite to eat with your friends? The Francophile crêperie La Galette, in an alley off Larsbjørnstræde, would be a good choice. A paper-thin buckwheat pancake with various fillings can be found on the menu for around DKK 65.

Thai

Thai Esan is a minor chain of Thai restaurants primarily in the district of Vestebro. The oriental kitchen is spicy and very varied, so if that's your taste, you will find several outlets.

Check kitsch

Restaurant Gold Prag in Gothersgade was chosen as a favourite budget place by the Copenhageners in a recent survey. Large, healthy helpings of Gullasch or pork chops are served.

Burger King
Rådhuspladsen 55, København V
Phone: 33 11 12 55

Restaurant Pizza Pasta
Vesterbrogade 31, København V
Phone: 33 24 42 23

Burger Palace
Vesterbrogade 85, København V
Phone: 33 23 26 82

Promenaden i Tivoli
Vesterbrogade 3, Tivoli, København V
Phone: 33 75 07 70

Taste of China
Vesterbrogade 114, København V
Phone: 33 23 05 76

American Pizza & Grill Bar
Gyldenløvesgade 13, København V
Phone: 33 12 10 13

McDonald's Familie-Restaurant
Banegårdspladsen 7, København V
Phone: 33 11 81 88

Astor Burger
Vesterbrogade 7, København V
Phone: 33 14 90 14

BEST OF THE BEST

Who else but the Copenhageners themselves should you ask, where to go for the just-right-meal? Every year a survey is carried out locally and based on the answers a 'Best Of' list is complied. Below you can see the winners:

Best Asian:

Yans Wok
Bagerstræde 9
1617 København V

Best Design Restaurant:

Langeliniepavillonen
Langelinie
2100 København Ø

Best Classical Danish Lunch Restaurant:

Nyhavns Færgekro
Nyhavn 5
1051 København K

Best New Restaurant:

Little Venice
Sundkrogskaj 17
2100 København Ø

SPECIAL SETTINGS

Enjoying dinner is not only about food, and we all know that the surroundings in which we dine are just as important. If you are looking for something out of the ordinary, Copenhagen has a varied selection of restaurants, offering everything from Viking parties to designer dreams.

Nyhavn

Picturesque Nyhavn, where Hans Christian Andersen once lived, is the oldest part of the Copenhagen Harbour and leads off from the square, Kongens Nytorv. Today old wooden ships are moored along the quay, and during the summer months the many restaurants on the southern, 'sunny side' move tables oudoors, where you can enjoy your lunch while watching the changing scenery.
This used to be the sailors' red light district but has today become very much 'in'. On a warm summer's day, so many people gather here to see and be seen that it looks as though the quay will sink! Most of the buildings date back to 1700s, which adds to the atmosphere.

Gråbrødre Torv
Another scenic place is the small square off the main pedestrian street, Gråbrødre Torv, behind the church of the Holy Ghost. Under the vaults of one of Copenhagen's medieval monasteries are several restaurants offering open sandwiches, informal lunches as well as French gourmet evening meals.

Tivoli

In this fairy tale garden in the heart of Copenhagen, which is open from mid April to mid September and again from late November to 23 December, there are more than 38 restaurants. Between them they have everything from fast food to 5-star gourmet cuisine. After dark, thousands of lights illuminate the garden making it the ideal place to take your date for a romantic evening.

Gammel Strand

The name actually means 'old beach' as this was originally the Copenhagen waterfront. Until about fifty years ago fishihng ships sailed up to the quay unloading their catch and making this the city's open fish market. One single fish stall remains today. But the location has attracted a number of restaurants, which can be found in the basement of some of the 17th century houses.

Modern surroundings

Not every eating place in town is nostalgic and historic. The restaurant of the new Royal Danish Library has an excellent view of the harbour. It is named after our famous philosopher, Søren Kierkegaard. Or you may choose to have your meal in the New Opera House restaurant, which also overlooks the harbour. Here you are on the top floor and look across to the Amalienborg Palace and the Marble Church - quite breathtaking!

VEGETARIAN (those marked * are particularly good value for the money)

Riz Raz *
Kompagnistræde 20, København K
Phone: 33 15 05 75

Casablanca
Turesensgade 21, kld., København K
Phone: 33 15 72 62

Cafe Charlottenborg
Nyhavn 2, København K
Phone: 33 13 11 58

Den Grønne Kælder *
Pilestræde 48, København K
Phone: 33 93 01 40

Cascabel
Store Kongensgade 80-82, København K
Phone: 33 93 77 97

Restaurant Flow
Gyldenløvesgade 10, København K
Phone: 33 14 43 43

Estin Madbutik
Lille Strandstræde 13, kld., København K
Phone: 33 32 27 77

Govindas Restaurant

Nørre Farimagsgade 82, København K
Phone: 33 33 74 44

Samsara
Herluf Trolles Gade 5, kld.,
København K
Phone: 33 32 37 08

Atlas Bar *
Larsbjørnsstræde 18, København K
Phone: 33 15 03 52

Taste
Store Kongensgade 80-82,
København K
Phone: 33 93 77 97

Flowfood take away
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Phone: 33 14 43 43

Riz Raz *
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Phone: 33 32 33 45

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The Proceedings of the Conference will be published under the auspices of the International Society for the Study of Harmful Algae (ISSHA) and the Intergovernmental Oceanographic Commission (IOC of UNESCO).

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